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(54) Title: SELF-REGULATED APOPTOSIS OF INFLAMMATORY CELLS BY GENE THERAPY (57) Abstract <p>This invention relates to the therapeutic induction of apoptosis in activated inflammatory cells, or cells at a site of inflammation, by introducing into those cells a chimeric gene containing an apoptosis-inducing gene (AIG) driven by a promoter of an inducible gene activated in inflammation and a promoter enhancer such that the inflammatory cells are targeted. In one embodiment, the chimeric gene comprises at least one TNFα promoter enhancer attached to a functional copy of a minimal TNFα promoter and further attached to at least one copy of an apoptosis-inducing gene, wherein expression of the gene is driven by the TNFα promoter. Attachment can be direct, distal, proximal or combinations thereof. Example apoptosis-inducing genes include caspase 3, caspase 4, caspase 5, Granzyme B. Advantageously, the TNFα-AIG chimeric gene is expressed in only those cells producing the inflammatory cytokine, TNFα. In addition, the TNFα-AIG chimeric gene also sequesters inducible TNFα transcription factors, thereby reducing endogenous production of TNFα. The invention also relates to methods of making and using self-regulated apoptosis chimeric genes and pharmaceutical compositions containing them for treating inflammatory diseases.</p>		

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SELF-REGULATED APOPTOSIS OF INFLAMMATORY CELLS BY GENE THERAPY

Related Application Data

This application claims priority benefit of co-pending U.S. provisional application serial number 60/039,266, filed February 28, 1997.

Technical Field of the Invention

- 5 This invention relates to the therapeutic induction of apoptosis in inflammatory cells by introducing into those cells a gene which induces apoptosis (programmed cell death or non-necrotic cell death) in these cells. The Apoptosis-Inducing Gene (which will sometimes be referred to herein as AIG) is driven by a TNF α promoter (TNFp) or other inducible gene activated in inflammation.
- 10 In one embodiment, apoptosis is selectively induced in those cells capable of producing TNF α . The TNFp-AIG or other chimeric gene may be conveniently introduced *in vivo* using conventional gene therapy techniques. Advantageously, in the embodiment wherein the chimeric gene is TNFp-AIG, it is expressed in only those cells producing the inflammatory cytokine, TNF α . In addition, since
- 15 the TNFp-AIG chimeric gene contains the TNF α promoter elements, it also sequesters inducible, TNFp-selective transcription factors. Such sequestration results in a reduction in endogenous production of TNF α . The present invention relates specifically to TNFp-AIG and similar gene constructs, cells containing chimeric genes, methods for induction of apoptosis in cells transfected with
- 20 chimeric genes, pharmaceutical compositions containing chimeric genes, methods

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for *in vitro* selection of TNF α non-producer somatic cell variants within a TNF α producing cell population and the like, a method for identifying dominant negative/dominant suppressive genes responsible for inhibiting TNF α production and therapeutic methods using the chimeric gene.

5 Background of the Invention

In many inflammatory conditions, cytokines such as IL-1, IL-10, GM-CSF and TNF α are excessively produced as a result of mass aggregation and accumulation of inflammatory cells (Brennan F.M. *et al.*, *British Medical Bulletin* 1995, 51/2, 368-384). Upregulation and/or dysregulation of cytokines in inflamed
10 tissue may be directly or indirectly responsible for exacerbation of chronic inflammatory diseases. For example, the most marked pathology in rheumatoid arthritis (RA) is displayed at the local site of inflammation (*i.e.*, the synovial joints). Therefore, it is likely that the cytokines produced in the synovial joints of RA patients play an important role in the disease process. Of those cytokines,
15 IL-1 and TNF α are believed to be responsible for the devastating cartilage destruction and bone erosion which characterizes RA (Dayer J.M. *et al.*, *J. Exp. Med.*, 1985, 162, 1208-1215; Gowen M. *et al.*, *Nature*, 1983, 306, 378-380). The presence of excessive amounts of IL-1 and TNF α in the synovial joints has
20 been shown to accelerate development of collagen-induced arthritis in rodents (Brennan F.M., *et al.*, *Clin. Exp. Immunol.*, 1994, 97/1, 1-3). Excessive amounts of TNF α and IL-1 are produced in the synovial tissue by a variety of cell types at the cartilage-pannus junction, including cells of the macrophage lineage, macrophage-like synoviocytes, activated T-cells and possibly fibroblast-like synoviocytes (Chu C.Q. *et al.*, *Arthritis & Rheumatism*, 1991, 34, 1125-1132;
25 Deleuran B.W., *et al.*, *Arthritis & Rheumatism*, 1992, 35, 1170-1178).

In addition to the above described inflammatory effects, TNF α plays a ubiquitous and key role in a variety of pro-inflammatory events, such as induction

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of IL-1 activity in monocytes. Indeed, anti-TNF α neutralizing antibodies have been shown to reduce overall IL-1 production (Portillo, *et al.*, *Immunol.*, 1989, 66, 170-175; Brennan F.M., *et al.*, *British Medical Bulletin* 1995, 51/2, 368-384). Thus, an added benefit to blocking the effect of the inflammatory cytokine TNF α is the reduction in production of the equally destructive pro-inflammatory mediator, IL-1. Furthermore, it is well known that TNF α is a transcriptional activator of other inflammation-related genes. For example, the presence of TNF α stimulates production of other cytokines (such as GM-CSF) and cell surface receptors, including HLA class II antigens and adhesion molecules (Alvaro-Garcia J.M., *et al.*, *J. Exp. Med.*, 1989, 146, 865-875), which results in continuous recruitment of activated T cells and neutrophils resulting in synovial inflammation and hyperplasia and ultimately, in augmented destruction of cartilage and bone (Allen J.B., *J. Exp. Med.*, 1990, 171, 231).

Conventional therapy against inflammatory disorders is typically directed against symptomatic inflammation. Such therapies provide only temporary relief without significantly delaying disease progression. In contrast, therapies targeting TNF α and other factors induced in the inflammatory process are likely to be more promising. For example, in collagen-induced arthritis animal models, an anti-TNF α antibody and soluble TNF α receptor-IgG chimera effectively reduced paw swelling, joint involvement and cartilage and bone destruction (Williams R.O. *et al.*, *Proc. Natl. Acad. Sci.*, 1992, 89, 9784-9788). Human trials using both humanized anti-TNF α antibodies and TNF α receptor-IgG chimeric molecules produced dramatic results (Elliott M.J., *et al.*, *Arthritis and Rheumatism*, 1993, 36, 1681-1690; Elliott M.J., *et al.*, *Lancet*, 343, 1105-1110). Although treatment with these TNF α antagonists appears to be well tolerated, it also results in production of antibodies against the recombinant proteins. Thus, these therapies may not be suitable for long term treatment and do not achieve true disease abatement. In order to actually modify progression of the disease, TNF α must be continuously targeted using TNF α -specific therapies. Such a therapeutic protocol

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is impractical with these biologic agents and would be difficult to administer in the long term.

In an alternate therapeutic option, inflamed synovium may be removed using surgical (Herold N. and Schroder H.A., *Acta Orthop. Scand.*, 1995, 66, 252-254; Ogilvie-Harris D.J. and Weisleder L., *Arthroscopy*, 1995, 11, 91-95), chemical (Cruz-Esteban C. and Wilke W.S., *Bailliere's Clinical Rheumatol.*, 1995, 9, 787-801) or radiation-induced synovectomy (Cruz-Esteban C. and Wilke W.S., *Bailliere's Clinical Rheumatol.*, 1995, 9, 787-801). The results following arthroscopic surgical synovectomy are good, showing improvement from the preoperative condition to the postoperative condition. Non-surgical synovectomy is performed using various chemical agents such as osmic acid, alkylating agents such as nitrogen mustard and thiotepa, methotrexate. Unfortunately, non-surgical synovectomies (including chemical and radiation-induced) are procedurally complicated, provide only short term relief and show only patchy reduction of the synovial hyperplasia. Furthermore, most of the non-surgical alternatives are potential teratogens. In addition, the chemical damage to afflicted tissue in non-surgical synovectomy, as well as surgically-induced tissue damage, often cause an inflammatory response themselves. Finally, it should be noted that these approaches suffer from the risks and side-effects commonly associated with conventional pharmaceutical therapy and invasive surgical procedures, including the expense and inconvenience of hospitalization and rehabilitation.

Accordingly, a need still exists for an effective therapeutic approach to treating inflammatory disorders in general and RA in particular.

Summary of the Invention

This invention overcomes the drawbacks associated with previous therapies for treating inflammatory disorders by providing a novel therapeutic

approach. According to one embodiment of this invention, apoptosis is selectively induced in TNF α -producing inflammatory cells, causing destruction of these cells without an associated inflammatory reaction.

One objective of this invention is to provide a therapeutic method
5 comprising the step of introducing into the inflammatory cells of a mammal, or cells at a site of inflammation, a chimeric gene containing a self-regulating apoptosis-inducing gene (AIG). The AIG is driven by a promoter such as a TNF α promoter (TNFp; see Figures 1 and 2), and, preferably, a promoter enhancer. Therefore, it is expressed in all and only those cells capable of producing TNF α .

10 Another objective of this invention is to provide TNFp-AIG and the like chimeric gene constructs, processes for making them, methods of using them, and preparations containing them.

Yet a further objective of this invention is to provide a method for the induction of apoptosis in cells transfected with the TNFp-AIG chimeric gene, a
15 method for the *in vitro* selection of TNF α non-producer somatic cell variants in a population, a method for identifying dominant/negative genes responsible for the genesis of a TNF α non-producing population and a method for identifying products responsible for regulation of TNF α production (Figure 10).

These and other objectives will be readily appreciated by those of
20 ordinary skill in the art based upon the following detailed disclosure of the invention.

Brief Description of the Figures

Figure 1 is a schematic representation of TNFp-AIG chimeric genes of this invention. Apoptosis Inducing Gene (AIG) could be any, but not limited to,
25 the genes listed, viz., Caspases 1 to 10, Granzyme B, FasLigand, *etc.*

Figure 2 is a schematic drawing depicting the results of gene therapy using a TNFp-AIG chimeric genes of this invention.

Figure 3 is a summary of deletion constructs used for identification of the inducible cis elements of the TNF α promoter using luciferase gene (Luc) expression as the reporter system.

Figure 4 (a and b) provide a summary of results obtained using the constructs described in Figure 3. Transient expression of the constructs was assessed in two different TNF α -producing cell lines, viz., Jurkat (Figure 4a) and THP-1 (Figure 4b). Histograms in each figure show stimulation index as a measure of inducibility by activating agents such as PMA (Figure 4a) or LPS (Figure 4b) for individual experiments. The line superimposed in each figure indicates the mean inducibility averaged from 4 to 6 experiments.

Figure 5 is a flow chart for preparation of the TNFpAIG using selected native elements of the TNF α promoter and prodomain-deleted AIGs (AIGs used are Caspase and Caspase 4/5).

Figure 6 (a, b, and c) provide a summary of results from representative experiments performed to see expression of the chimeric TNFpAIGs. Apoptosis in transiently-transfected Jurkat cells (Figure 6a and 6b) and THP-1 (Figure 6c) cells was assessed using Cell Death Elisa (CDE assay). In all three figures, histograms with sparse dots represent transfection control, where cells were treated with the transfecting agent in the absence of DNA. Histograms with dense dots represent the TNFp elements driving expression of the luciferase gene and solid histograms represent the same TNFp elements driving expression of either AIG.1 or AIG.2. The number in parenthesis above the solid histograms represent enrichment factor (ratio of apoptosis induced by TNFpAIG to the TNFpLuc control vector).

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Figure 7 (a and b) is a diagrammatic representation of a TNFp-AIG chimeric gene of this invention, comprising multiple copies of the inducible cis elements of the TNF α promoter which, in turn, drive expression of the AIG (Figure 7a). A diagrammatic representation of a TNFpAIG chimeric gene, comprising multiple copies of the inducible cis elements of the TNF α promoter, driving expression of the AIG, downstream of which are 3' untranslated region of the TNF α gene (TNF3'UTR) (Figure 7b). 3'UTR of the TNF α gene is implicated in the regulation of the inducible expression of TNF α (Han, J., *et al.*, *J. Immunology*, 1991, 146, 1843-1843, Crawford, E.K., *et al.*, *J. Biol. Chem.*, 1997, 272, 21120-21137, and Figure 9).

Figure 8 (a and b) are flow charts of schemes for preparing TNF α superpromoter-AIG chimeric constructs.

Figure 9 shows a summary of the results of two experiments to show the regulatory effect of the TNF3'UTR on inducible expression of the luciferase reporter gene. The transient transfection was performed in a fibroblast cell line. Dotted histograms represent inducibility of TNFpLuc in the absence of TNF3'UTR and solid histograms represent inducibility of TNFpLuc in the presence of TNF3'UTR. Similar results are obtained in the Jurkat cell.

Figure 10 is a diagrammatic representation for the selection of TNF α non-producer somatic cell variants within a TNF α -producing cell population and identification of dominant negative suppressive genes responsible for inhibiting TNF- α production.

Detailed Description of the Invention

This invention is based upon evidence that apoptosis of inflammatory cells in certain inflammatory diseases is therapeutically beneficial. The invention

specifically relates to self-regulated apoptosis by gene therapy. Broadly speaking, in the practice of the invention, a chimeric gene comprising at least one promoter enhancer attached to at least one functional copy of a minimal promoter, the promoter being a gene or combination of genes activated in inflammatory cells or
5 in cells at a site of inflammation, is attached to at least one copy of an apoptosis-inducing gene (AIG), such that the expression of the apoptosis-inducing gene is driven by the promoter, thus targeting the inflammatory cells. Example promoters of inducible genes activated in inflammation include, but are not limited to, cytokines, interleukins and their receptors, cell adhesion molecules and their
10 ligands, chemokines and their receptors, pro-inflammatory enzymes, and the like. Chimeric genes according to the invention comprise enhancer, promoter, and AIG elements in direct, distal, or proximal attachment, and combinations thereof. As mentioned above and will be discussed in more detail below, in some embodiments, multiple copies of the enhancer, promoter, and/or AIG are employed for
15 maximal efficacy.

In order that the invention herein described may be more fully understood, the following detailed description is set forth, with emphasis on chimeric genes comprising at least one TNF α promoter enhancer attached to at least one functional copy of a minimal TNF α promoter and further attached to at least one
20 copy of an AIG for illustrative purposes only. Though the examples that follow also employ these types of constructions, it will be appreciated by skilled workers that the basic constructs described herein may be altered to provide other embodiments that utilize products, processes, methods, and compositions of the invention with other promoters comprising inducible genes activated in inflammation such as
25 the types listed above that exhibit similar functions that can be used to target cells at the site of infection.

For example, cytokines and interleukins useful as promoters in the construction of chimeric genes of the invention include, but are not limited to, TNF- α , TNF β , IL-1 α , IL-1 β , IL-2, IL-6, IL-9, GM-CSF, interferon γ , and the

like, and functional fragments and mixtures thereof. Cell adhesion molecules and their ligands include, but are not limited to, selectins, integrins, and members of the immunoglobulin superfamily such as ICAM-1, V-CAM, and the like, and functional fragments and variants and mixtures thereof. Chemokines and their
5 receptors include, but are not limited to, the C-X-C and C-C family members such as MIP-1 α , MIP-1 β , MCP1-4, RANTES, Mig, NAP2, IP10, Gro α - γ and the like, and functional fragments and variants and mixtures thereof. Pro-inflammatory enzymes include, but are not limited to COX-2, iNOS, phospholipases, proteases (including matrix metalloproteases), and the like and functional fragments and
10 mixtures thereof.

To clarify the discussion below of exemplary TNFp-AIG chimeric genes of this invention, the following sequences are illustrated:

SEQ ID NO: 1 is the nucleotide sequence corresponding to the full-length, reference human TNF α promoter sequence, as published in (Takashiba
15 S., *et al.*, *Gene*, 1993, 131, 307-308). Nucleotide numbers used herein refer to the numbering of this sequence.

SEQ ID NO: 2 is the native TNF α promoter sequence of the gene that was used in this invention (-1077 nucleotides from the transcription start site, TSS). There are a few differences in the sequence of the TNFp in SEQ ID NO: 1
20 and SEQ ID NO: 2. Such differences in the nucleotide sequences of the TNF α promoter have been reported (Takashiba S., *et al.*, *Gene*, 1993, 131, 307-308).

SEQ ID NO: 3 is the native minimal TNF α promoter sequence (nucleotide -120 through -TSS, which includes at least one enhancer element (kl site; see
25 Pauli, U., *Crit. Rev. in Eucaryotic Gene Expression*, 1994, 4, 323-344; Rhoades K.L., *et al.*, *J. Biol. Chem.*, 1992, 267, 22102-22107; and Takashiba S., *et al.*, *Gene*, 131, 307-108).

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SEQ ID NO: 4 is the chimeric gene TNFp120 AIG.1 (containing -120 TNFp driving the expression of the prodomain-deleted variant of CPP32 gene (Caspase 3, published Tewari M. *et al.*, *Cell*, 1995, 81(5), 801-809, with the variation being V239A).

5 SEQ ID NO: 5 is the chimeric gene TNFp706 AIG.1 (containing -706TNFp driving expression of the prodomain-deleted CPP 32 gene.

SEQ ID NO: 6 is the TNFp1005 AIG.1 (containing -1005 TNFp driving expression of the prodomain-deleted CPP 32 gene).

10

SEQ ID NO: 7 is the chimeric gene TNFp120 AIG.2 (containing -120 TNFp driving expression of the prodomain-deleted Ty/x gene. (Sequences of Ty (Caspase 5) and Tx (Caspase 4) genes are published in the ref. Faucheu, C., *et al.*, *Eur. J Biochem.*, 236, 207-213, 1996; Faucheu, C., *et al. EMBO J.*, 14, 1914-1922, 1995).

15

SEQ ID NO: 8 is the chimeric gene TNFp706 AIG.2 (containing -706TNFp driving expression of the prodomain-deleted Ty/x gene.

20

SEQ ID NO: 9 is the TNFp1005 AIG.1 (containing -1005 TNFp driving expression of the prodomain-deleted Ty/x gene).

SEQ ID NO: 10 is the enhancer region 1 (ER1) of the TNF α promoter encompassing nucleotides -1005 to -905.

25

SEQ ID NO: 11 is the enhancer region 2 (ER2) of the TNF α promoter encompassing nucleotides -706 to -517.

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SEQ ID NO: 12 is additional multiple cloning sites (MCS) genetically engineered upstream of the -120 minimal TNF α promoter in the -120pGL3 construct.

SEQ ID NO: 13 is the 3' untranslated region (3'UTR) of the TNF α gene (Nedwin, G.E., *et al.*, *Nucleic Acid Research*, 1985, 13, 6361-6373).

5 The elements of the TNF α promoter for preparation of chimeric gene constructs according to this invention are selected from elements which are capable of inducing expression of a therapeutic gene driven by the TNF α promoter. These promoter elements will be referred to herein as "inducible cis elements", "cis-inducible elements" or "enhancer elements" of the TNF α promoter.

10 The enhancer elements may be physically linked to the minimal promoter sequence, or separated from the minimal promoter by a linker sequence which may or may not have unique restriction sites. Thus, as summarized above, enhancer elements may be attached directly, distally, proximally, or any combination thereof, to chimeric genes of the invention. These are typically constructed
15 upstream of the promoter. Example TNF α enhancer elements are set out in SEQ ID NO: 10 and SEQ ID NO: 11; functional fragments or variants and combinations thereof may be employed. Some preferred gene constructs according to this invention include those that have multiple copies of the enhancer elements, *i.e.*, 2 or more copies. Some embodiments have about 2 to 25, more narrowly 2 to 10,
20 and even more narrowly, 2 to 5 copies.

 The terms "TNF promoter", "TNF α promoter" and "TNFp" are used interchangeably herein. Unless noted to the contrary, these terms refer to the entire nucleotide sequence corresponding to a native TNF α minimal promoter sequence attached to one or more upstream enhancer elements (either present
25 naturally *i.e.* native, or genetically engineered in the laboratory). Examples include, but are not limited to, SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, and functional fragments, variants, and mixtures of any of these. Many

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functional fragments and variants of these TNF α sequences and others described herein share a sequence homology of at least about 80%, and in some cases over 90%, to their native and genetically engineered counterparts, but these are known to skilled workers and defined in the references cited herein.

5 Any apoptosis-inducing gene may be used in the chimeric genes and methods described herein. The apoptosis inducing gene used for the chimeric therapeutic genes of this invention can be the same or different from the type of apoptosis inducing gene present in the native sequence of the TNF- α -producing inflammatory cells (if those cells naturally contain an apoptotic gene). Preferred
10 AIGs include, but are not limited to, members of the ICE/CED3 family of apoptosis inducing proteases (such as Caspase-1 (ICE), hICE, ICE-LAP45, Mch2 α), Caspase-2 (ICH1), Caspase-3 (CPP32, Yama, Apopain), Caspase-4 (TX, ICH2, ICE rel II), Caspase-5 (ICE rel III, TY), Caspase-6 (Mch-2), Caspase-7 (Mch-3, ICE-LAP3, CMH-1), Caspase-8 (MACH, FLICE, Mch-5), Caspase-9
15 (ICE-LAP6, Mch6) and Caspase-10 (Mch4)), members of the granzyme family (such as Granzyme A and Granzyme B), Fas ligand (FasL), and functional fragments, variants, and mixtures of any of these. Some embodiments employ Caspase 3, Caspase 4, Caspase 5, Granzyme B, and functional fragments, variants, and mixtures thereof. With the exception of FasL, these genes, when
20 overexpressed following transfection, induce apoptosis in the transfected cells (Miura M., *et al.*, *Cell*, 1993, 75, 653-660; Chinnayan A.M., *et al.*, *Cell*, 1995, 81, 505-512; Los, *et al.*, *Nature*, 1995, 375, 81; Muzio, *et al.*, *Cell*, 1996, 85, 817-827).

 In the case of FasL, apoptosis is induced (either in an autocrine or a
25 paracrine fashion) in only those cells that express Fas. Therefore, the TNFp-FasL chimeric gene construct offers a second level of selectivity. Another advantage of the TNFp-FasL chimeric gene is the selective targeting of those disease-producing cells in the synovium that do not express TNF α (thereby failing to drive expression of the apoptosis inducing gene), but do express Fas on the surface. In this

case, FasL will be expressed by the cells that are capable of producing TNF α such as activated macrophages and T cells. These cells will then induce apoptosis in Fas-expressing cells such as hazardous activated T cells and Fas-expressing synoviocytes.

5

This invention provides a novel therapeutic method comprising the step of introducing into the cells of a mammal a chimeric gene comprising an apoptosis-inducing gene (AIG) driven by the TNF α promoter (TNFp). Example chimeric genes of the invention are set out in SEQ ID NOs 4, 5, 6, 7, 8, and 9; functional fragments or variants of these may also be employed. Without wishing to be bound by theory, as a result of being controlled by the TNFp, AIG is expressed in only those cells producing the inflammatory cytokine, TNF α . Therefore, any cells expressing TNF α will be self-destructive, while cells that do not express TNF- α will be unaffected. Advantageously, this methodology can target any TNF α -producing cells (such as activated macrophages, activated T-cells and macrophage-like and possibly fibroblast-like synoviocytes) without regard to cell type. Indeed, the targeted TNF α -producing cell can be one which normally does or normally does not carry or expresses an apoptosis gene in its native, unaltered form. Therefore, using the chimeric genes and methods of this invention, the cellular sources of TNF α can be destroyed in a highly selective manner.

Another advantage of using the TNFp-AIG chimeric gene of this invention is that TNFp sequesters transcription factors needed by endogenous TNFp, thereby leading to a reduction in endogenous TNF α production. In one preferred embodiment, TNFp is present in the therapeutically targeted cell in vast excess. This may be accomplished by introducing multiple copies of the transfected gene into the cell. Alternatively, the TNFp-AIG chimeric gene according to this invention can contain multiple copies of the inducible cis elements of the TNF α promoter. As mentioned above, multiple copies of the "inducible enhancer elements" of TNFp are present in some embodiments of the TNFp-AIG chimeric genes of this invention. By including multiple copies of the inducible cis elements

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of the TNFp construct, the transcriptional factors needed by the transfected cell to produce TNF α are sequestered by the exogenously introduced sequence. This preferred chimeric TNFp-AIG construct is characterized by an increased effectiveness in competing for the TNFp-specific transcription factors as compared to
5 chimeric genes of this invention containing only a single enhancer element linked to TNFp. The "inducible super promoter" constructed in this way is capable of (1) more effectively competing for TNF α specific inducible transcription factors and (2) driving expression of the apoptosis inducing gene in an augmented fashion by virtue of multiple enhancing elements.

10 For example, in rheumatoid arthritis patients, synovectomy, *i.e.*, removal of synovial tissue, has been shown to be clinically beneficial. Unlike conventional and surgical synovectomy procedures, the cell-targeted therapeutic method described herein targets only cells producing TNF α . Thus, advantageously, the introduction and expression of the TNFp-AIG chimeric gene, and
15 subsequent induction of apoptosis do not induce an inflammatory response. Accordingly, methods of this invention are comparatively selective and result in minimal tissue damage and a reduction in inflammation.

The products and methods described herein are useful for the treatment of other inflammatory disorders as well. Such inflammatory disorders include, but
20 are not limited to, multiple sclerosis, Guillain-Barre syndrome, Crohn's disease, ulcerative colitis, psoriasis, graft versus host disease, lupus erythematosus, insulin-dependent diabetes mellitus, psoriatic arthritis, sarcoidosis, hypersensitivity pneumonitis, ankylosing spondylitis and related spondyloarthropathies, Reiter's syndrome and systemic sclerosis. Thus, this invention encompasses methods for
25 treating an inflammatory disorder in a patient by inducing apoptosis in inflammatory cells or cells at a site of inflammation of the patient by introducing into the cells at least one chimeric gene of the invention. This is typically accomplished by preparing a pharmaceutical composition containing at least one chimeric gene of the invention and typically a pharmaceutically acceptable carrier, and administer-

ing the composition to a patient using standard means. In some embodiments, the pharmaceutical composition is delivered directly to the site of inflammation using local topical, intravenous, intraperitoneal, and similar methods. Further methodology is discussed below.

5 In addition to the therapeutic indications, the genes and cells according to this invention can be used in a variety of useful screening and selection methods. In one such method, TNF α non-producer somatic cell variants within a TNF α producing cell population can be selected in vitro by introducing a TNFp-AIG chimeric gene into the TNF α producing cell population. Cells
10 producing TNF α will undergo apoptosis. Cells that do not produce TNF α will survive. Selection of those cell variants possessing the survival phenotype is an easy way to identify TNF- α non-producer cells. Such a selection process may be used to determine expression of genes that act in-trans to regulate activity of the TNF- α promoter, thereby reducing TNF- α production. Such genes are character-
15 ized as dominant negative (DN) /dominant suppressive genes in other systems (Behrends S., *et al.*, *J. Biol. Chem.* 1995, 270, 21109-21113; Zhang S., *et al.*, *J. Biol. Chem.*, 1995, 270, 23934-23936; Watowich S.S., *et al.*, *Mol. Cell Biol.*, 1994, 14/6, 3535-3549).

 In a further *in vitro* method, a TNFp-AIG chimeric gene according to
20 this invention can be used to identify dominant negative genes responsible for the genesis of a TNF α non-producing cell population. According to this method, a TNFp-AIG chimeric gene according to this invention is introduced into cells that produce TNF α . Barring the presence of a dominant negative gene, those cells should undergo apoptosis upon activation. Therefore, it can be deduced that
25 surviving variants possess a dominant negative gene capable of down-regulating TNF α production. The dominant negative gene can be readily identified by producing a cDNA library and transfecting cell lines (*e.g.*, Jurkat and THP-1). These cells are either stable transfectants of an inducible TNFp-AIG chimeric gene or TNFp-luciferase gene TNFp-AIG transfected cells will be selected for the

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survival phenotype following *in vitro* activation; survival phenotype is indicative of the effect of the DN genes. In the cells transfected with TNFp-luciferase gene, reduction in the luciferase activity will be indicative of the DN gene effect. Dominant negative genes identified using this protocol can be used as the future therapeutic agents themselves. Such genes will be the candidates for gene therapy in order to reduce TNF α production.

The methods utilized for gene transfer are grouped into two broad categories:

1. Direct approach : *In situ* transduction of the therapeutic gene into target cells such as synoviocytes using a suitable vector as a carrier for the therapeutic gene. The vector containing therapeutic gene is injected directly into the affected area (*e.g.*, an arthritic joint).
2. Indirect approach : *Ex-vivo* transfection of the therapeutic gene into target cells such as synoviocytes. In this approach, the synovium is removed from joints, synoviocytes are isolated and cultured *in vitro*. *In vitro* cultured cells are transfected with the therapeutic gene, and genetically modified synoviocytes are transplanted back into the synovium.

For *in vivo* transfer, several vectors have been evaluated for their efficacy in gene delivery (Nita, *et al.*, *Arthritis & Rheumatism*, 1996, 39/5, 820-828). Among the vectors used for gene therapy, the vectors derived from retroviruses are by far the best developed. They are able to insert genetic material in the host genome and produce stable transfectants. These vectors, however are unable to infect non-dividing cells and, since they are inserted in the host genome, the possibility of insertional mutagenesis cannot be ruled out. In comparison, the vectors derived from adenoviruses infect dividing as well as non-dividing cells and deliver DNA episomally. The disadvantage of adenovirus based vectors is that these vectors continue to produce viral proteins in infected cells making them

potentially antigenic. A third type of viral based vectors is derived from Herpes simplex viruses (HSV), which are also capable of infecting dividing as well as non dividing cells.

Among the non-viral vector systems, cationic liposomes and naked
5 plasmid DNA have been evaluated. Liposomes are at the most advanced stage of development, although certain types of cells such as muscle and skin take up, retain and express naked plasmid DNA.

Particle-mediated gene-delivery system is also possible (Rakhmilevich, *et al.*, *PNAS*, 1996, 93, 6291) and is a promising approach.

10 The following "*in vivo*" gene delivery protocols can be used to deliver the chimeric genes of this invention:

(1) Nita *et al.*, *Arthritis and Rheumatism*, 1996, 39, 820-823

In vivo experiment in rabbits:

Each vector is injected intra-articularly into 1 knee joint. For viral vectors,
15 between 10^8 and 10^9 particles suspended in 0.5 ml balance salt solution are injected per knee.

Liposome-DNA complexes (200 nmoles of DC-Chol complexed with 20 μ g of DNA/ml) in 1 ml balance salt solution are injected per knee.

(2) *Methods in Molecular Medicine : Gene Therapy Protocols*, Paul Robbins, ed.,
20 1997, Barr *et al.*, pages 205-212

Adenovirus-based vector delivery to hepatocytes : Rat hepatocytes
 1×10^{11} PFU in 100 g animal.

In dogs (12-17 kg), portal vein is perfused with about 1.5×10^{11} PFU/kg
gives 1 adenovirus genome copy per diploid copy of host DNA

25 In rabbits (2-4kg), 1.5×10^{13} virus particles (about 1.5×10^{11} PFU) gives 100% hepatocyte transduction; 4×10^{12} virus particles give 50-75% transduction.

Yang N-S, *et al.*, 281-296

Gold particle-mediated gene delivery : Transfection of mammalian skin tissue- 0.1, 0.5, 1.0 and 2.5 μg of DNA/mg particle gives linear relationship with transgene expression levels.

5 Nabel, *et al.*, 297-305

Liposome-mediated gene delivery in humans:

Protocol 1: 15nmol DC-Chol/Dope liposomes combined with 1 μg DNA in 0.7 ml. 0.2 ml of the above mixture is injected into the patient's melanoma nodule. For catheter delivery, 0.6 ml of
10 the solution is delivered into the artery.

Protocol 2: 15nmol DMRIE/Dope liposomes combined with 5 μg DNA in 1.0 ml.

For direct intra-tumor injections, DNA concentrations range from 3 μg complexed with 4.5nM DMRIE/Dope to 300 μg complexed with 450 nM

15 DMRIE/Dope.

(3) Roessler, *et al.* 369-374

Gene transfer to synovium:

A range of doses, 10^9 - 10^{12} adenovirus particles containing therapeutic gene/joint are used... However, the optimal dose for any
20 particular experimental series needs to be determined empirically, and is dependent on both the properties of the recombinant adenoviral genomic backbone being used as well as the transgene being expressed.

For the indirect approach, a variety of methods are well established,
25 including utilization of cationic lipid or cationic polymer-based transfection and electroporation.

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Any of the above-referenced techniques can be altered to suit the particular needs of those of ordinary skill in the art. Such modifications are well within the level of skill possessed by ordinary practitioners and do not require undue experimentation. These obvious variations are within the scope of this invention.

Examples

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustrating some preferred embodiments of this invention, and are not to be construed as limiting the scope of this invention in any way.

EXAMPLE 1

Production of TNFp-AIG Constructs

In order to construct chimeric AIG driven by the enhancer cis elements of the TNF promoter, either in a single or multiple copies of the same region or various regions, identification of the regions of interest responsible for optimal inducible expression of the reporter gene is performed.

Selection of the TNF- α promoter elements for constructing a chimeric gene. The regions of the TNF- α promoter are amplified by polymerase chain reaction (PCR) using primers encompassing various deletion constructs of the TNF α promoter (Figure 3). The regions identified by other investigators in various other cellular systems are used as reference (Rhoades, *et al.*, *J. Biol. Chem.*, 1992, 267, 22102-22107; Leitman, *et al.*, *Mol. Cell Biol.*, 1992, 12, 1352-1356; Pauli U., *Crit. Reviews Eukaryotic Gene Expression*, 1994, 4, 323-344). The PCR-amplified genes are then cloned upstream of a reporter gene, such as luciferase, in a commercially available promoterless vector. These

constructs are tested for their constitutive and inducible expression in various cell lines such as Jurkat (T lymphoblastoid), U937 (myelomonocytic), THP-1 (monocytic), fibroblasts and in vitro cultured human synoviocytes. Identification of the regions responsible for inducible expression of the reporter gene is primarily based on the results obtained using two TNF α -producing cell lines, viz Jurkat (following stimulation with PMA) and THP-1 (following stimulation with LPS) (Figure 4 a and b). These cells are transiently transfected by using well established methods and commercially available reagents, *e.g.*, DEAE dextran and Superfect. The cis-elements of the TNF α promoter that are responsible for inducible expression of the reporter gene are then used for constructing TNFp-AIG chimeric genes.

Construction of TNFp-AIG chimeric genes. Of the apoptosis inducing genes described herein, the following genes are preferred:

- i) cysteine protease - CPP32 (also known as Yama, apopain or Caspase 3) and
- ii) Cysteine protease -Tx/Ty (Caspase 4/Caspase 5)

The AIGs are used as "prodomain-deleted"truncations in order to potentially augment autocatalysis of Caspases. This is essential for conversion of inactive Caspase to active form.

Prodomain-deleted CPP32 is amplified using primers corresponding to codons 29-36 and 271-278 (278 is a stop codon). The truncated form of CPP32 is referred to as " α CPP32" or "AIG.1" herein.

For PCR amplification for prodomain-deleted Ty, primers corresponding to the sequences in the Ty gene are synthesized. All Caspases discovered so far have homology to the other members of the Caspase family. The 3' primer corresponding to the codons 359-365 (codon 365 is a stop codon) shares 100% sequence homology to the codons 372-378 (codon 378 is a stop codon) in the Tx gene. However, the 5' primer corresponding to codons 81-87 in the Ty gene does not share 100% homology with the corresponding region in the Tx gene (Tx codons 94-100). Residue 87 (Alanine) in the Ty gene differs from residue 100 (Glycine) in the Tx gene. The PCR amplified product generated from cDNA prepared from activated human peripheral blood lymphocytes possesses the

sequence of Tx, due to apparent abundance of Tx transcripts. Therefore, the truncated form of the AIG generated using synthetic oligonucleotide primers corresponding to the sequences in Ty, indeed matches sequences in Tx, albeit flanked by Ty sequences of the primers. The Ty sequences of the primers used
5 also match with the sequence of Tx, except for one codon. Thus the gene used in this invention matches truncated Tx gene with residue G100 to A change. This gene is referred to as "ΔTy/x" or "AIG.2" herein.

AIG.1 and AIG.2 are inserted downstream of the TNFα promoter by replacing the luciferase reporter gene in deletion constructs (-120, -706 and -1005)
10 of the TNFα promoter (Figure 5). These constructs are tested for the induction of apoptosis following stimulation of transiently-infected Jurkat and THP-1 cells (Figures 6 a, b, and c).

Construction of TNFα superpromoter-AIG chimeric genes. Two broad preferred regions, viz., ER1(-1005 to -905) (SEQ. ID 10) and ER2(-706 to -517)
15 (SEQ ID NO: 11) of the TNFα promoter, containing elements responsible for inducible expression of the reporter gene described above (Figure 4a and 4b) are PCR amplified and are ligated upstream of the minimal native promoter (-120 through TSS, SEQ ID NO: 3), either as a single copy or multiple copies. Two more regions (-234 to -120) and (-234 to -65) of the TNFα promoter is also
20 identified as a potential enhancer region 3 (ER3) and enhancer region 4 (ER4), respectively, which can be employed in the chimeric constructs using the strategies described below. The super promoter contains multiple (2-10) cassettes of the above mentioned regions containing inducible promoter elements (Figure 7). This is achieved by PCR amplifying the regions of interest using primers synthesized
25 with restriction sites inserted at the 5' end of each of the primers. These unique restriction sites flank the amplified gene product of interest. Preferably, PCR amplified AIG is cloned downstream of the TNF- (super promoter, replacing the luciferase reporter gene in the original construct as described (Figure 5) for the native TNFα promoter.

The schemes for construction of a TNF α superpromoter and the linker sequences representing unique restriction sites (these restriction sites are absent in the selected elements of the TNF- α promoter and the AIG in question) for efficient directional insertion is outlined below and depicted in Figure 8:

5 Scheme 1:

STEP 1: Insertion of the TNF- α minimal promoter (-120 to TSS) into the pGL3 basic (promoterless) luciferase vector (Promega):

The elements of pGL3 basic vectors that are used for construction of the chimeric gene TNFp-AIG are shown below.

10 _____KpnI.SacI.MluI.NheI.SmaI.XhoI.BglII.HindIII. [luciferase].XbaI _____

The minimal promoter is PCR amplified using primers containing XhoI and BglII.HindIII sites, so that XhoI is at the 5' end and BglII.HindIII sites are at the 3' end of the amplified product. This fragment is inserted into the polylinker of the pGL3 basic vector using these same restriction sites. This construct is

15 referred to as "Construct A1" and is as follows:

____KpnI.SacI.MluI.NheISmaIXhoI.(-120 to TSSBglII).HindIII.[luciferase].XbaI____

STEP 2: The enhancer fragment (ER1 or ER2) is PCR amplified using the primer containing several restriction sites. The resulting fragment will have restriction sites KpnI.AatII.BssHII at the 5' end and NsiI.SpeI.MluI at the 3' end as follows:

20 5' KpnI.AatII.BssHII.(ER1 or ER2).NsiI.SpeI.MluI 3". The fragment is inserted into the "Construct A1" generated in STEP 1 using KpnI and MluI restriction sites. This construct is referred to as "Construct B1" and is as follows:

____KpnI.AatII.BssHII.(ER1 or ER2).NsiI.SpeI.MluI.NheI.SmaI.XhoI(-120 to TSS BglII).HindIII. [luciferase].XbaI_____

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STEP 3: The TNF α enhancer fragment (ER1 or ER2)) is amplified using the primers containing restriction sites AatII and BssHII to generate the PCR product as follows:

5' AatII.(ER1 or ER2).BssHII 3'. This fragment is cloned into the "Construct B1" using these same restriction sites. This construct is referred to as "Construct C1" and is as follows:

_____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.SpeI.MluI.NheI
SmaI.XhoI(-120 to TSS BglII).HindIII.[luciferase].XbaI _____

STEP 4: The TNF α enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites NsiI and SpeI to generate the PCR product as follows:

5' NsiI.(ER1 or ER2).SpeI 3'. This fragment will be cloned into the "Construct C1" using these same restriction sites. This construct is referred to as "Construct D1" and is as follows:

15 _____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.(ER1 or ER2).SpeI.MluI.NheI SmaI.XhoI(-120 to TSSBglII).HindIII.[luciferase].XbaI

STEP 5: AIG.1 or AIG.2 (preferred but not limited to AIG.1 and AIG.2; any AIG from the list can be used) coding regions are PCR-amplified using the primers containing BglII and XbaI restriction sites generating the fragment as follows: 5' BglII.(AIG.1 or AIG.2).XbaI 3". This fragment is inserted into the "Construct D1" using these same restriction sites. The resulting construct is referred to as "Construct E1" and is as follows:

25 _____KpnI.AatII.(ER1 or ER2).BssHII.(ER1 or ER2).NsiI.(ER1 or ER2).SpeI.MluI.NheI.SmaI.XhoI(-120 to TSS.BglII)[AIG.1 or AIG.2].XbaI _____

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Alternatively scheme 2 is followed:

Scheme 2:

STEP 1: Same as in scheme I giving rise to "Construct A1", which is as follows:

KpnI.SacI.MluI.NheI.SmaI.XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI

5 STEP 2: Insertion of additional MCS.

Two complementary oligonucleotides (5' phosphorylated) providing _____NheI.SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.XhoI_____ are synthesized using commercial sources. These oligonucleotides are annealed and then cloned into NheI and XhoI sites of the "Construct A1". The resulting construct referred to as "Construct B2" and it is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

STEP 3: The TNF- α enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites SpeI.PvuII at the 5' end, and XhoI at the 3' end to generate the PCR product as follows: 5' SpeI.PvuII.(ER1 or ER2) . XhoI 3'. This fragment is cloned into the "Construct B2" using SpeI and XhoI restriction sites. This construct is referred to as "Construct C2" and is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI.PvuII.(ER1 or ER2) XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

20 STEP 4: The TNF α enhancer fragment (ER1 or ER2) is amplified using the primers containing restriction sites AvrII. SpeI at the 5' end, and PvuII at the 3' end to generate the PCR product as follows: 5' AvrII.SpeI.(ER1 or ER2).PvuII 3'. This fragment is cloned into the "Construct C2" using AvrII and

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PvuII restriction sites. This construct is referred to as "Construct D2" and is as follows:

_____KpnI.SacI.MluI.NheI. SacII.EcorV.AflII.AatII.AvrII.SpeI. (ER1 or ER2)
PvuII. (ER1 or ER2) XhoI.(-120 to TSS BglII).HindIII.[luciferase].XbaI_____

5 Thus, using this strategy at least seven copies of the enhancer regions (ER1, ER2 or ER3, individually or in combination), one at a time, can be added by using one more restriction site upstream of the previous one in PCR amplification of the enhancer regions of choice.

10 Once the desired number of copies of the enhancer regions are added, AIG is inserted downstream of the superpromoter as described in the STEP 5 of the scheme 1.

15 The inducible expression of the chimeric TNFp-AIG gene is tested by transient transfection of the cell lines mentioned above. The expression of TNFp-AIG gene is measured by detecting apoptosis of transfected cells, assessing AIG expressed proteins in Western blots using commercially available antibodies and assessing protease activity using commercially available, well documented specific synthetic tetrapeptide substrate.

20 The inducible expression of the chimeric TNFp-FasL gene is tested by transient transfection of the same cell lines. The cell surface expression of FasL by the transfected cells is quantitated using anti-FasL antibody binding as detected by indirect immunofluorescence and by measuring induction of apoptosis of Fas positive cells.

25 Regulation of the TNFp-driven expression of a reporter gene. The 3' untranslated region of the TNF α gene plays an important role in regulation of the TNF α biosynthesis. It is involved in translational expression of the TNF α gene in

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normal, non-activated states. Importantly, these elements allow de-repression to occur when TNF α -producing cells are activated by external stimuli (Han, J., *et al.*, *J. Immunology*, 1991, 146, 1843-1848; Crawford, F.K., *et al.*, *J. Biol. Chem.*, 1996, 271, 22383-22390).

- 5 Genetic constructs are made in which the entire 3' untranslated region (SEQ ID NO: 13) is inserted downstream of the luciferase gene driven by deletion fragments, viz., -120, -706 and -1005 of the TNF α promoter. The results of the transient expression of these constructs are summarized in Figure 9.

EXAMPLE 2

10 Testing Protocols

In Vitro Methods:

Luciferase assay: Luciferase activity is determined using commercially available reagents (Promega).

AIG.1 and AIG.2 gene expression:

- 15 a) Western blots of the transfected cell lysates are developed using anti-CPP32 antibody as well as anti-PRAP antibody. Anti-PRAP antibody detects both hydrolyzed as well as non-hydrolyzed products of PRAP as an enzymatic action of CPP32.
- 20 b) CPP32 enzyme assay : This assay detects enzymatic reaction of CPP32 and breakdown of colorimetric or fluorogenic substrate. Commercially available (Clonotech, Pharmingen) kit is used for this assay.
- 25 c) Apoptosis of transfected cells: Apoptosis of transfected cells due to AIG.1 and AIG.2 is determined by staining nuclei by propidium iodide (Krishan, A., *J. Cell Biol.*, 66, 1994, 188-193) and by commercially available Cell Death Elisa kit (Boehringer Mannheim).

Animal Models

Rabbit model of IL-1-induced arthritis (Pettipher E. R., *et al.*, *Proc. Natl. Acad. Sci.*, 1986, 83, 8749-8753): IL-1 is injected into the knee joints of New Zealand White rabbits. Intra-articular injection of IL-1 causes dose-dependent infiltration of leukocytes into the joint space and loss of proteoglycan from the articular cartilage.

Antigen-Induced arthritis : Intra-articular injection of antigen (ovalbumin) into knee joints induces leukocyte accumulation and cartilage degradation that closely resembles rheumatoid arthritis in humans. The joint swelling following the injection was sustained for 14 days.

Scid mice-human synoviocytes model (Houri J.M., *et al.* *Current Opinions in Rheumatol.*, 1995, 7, 201-205; Sack U., *et al.*, *J. Autoimmunity*, 1995, 9, 51-58; Geiler T., *et al.* *Arthritis & Rheumatism*, 1994, 37, 1664-1671): These are recently developed models for arthritis in which fresh synovial tissue from RA patients is implanted with normal human cartilage into scid mice either subcutaneously, under the renal capsule (Geiler T., *et al.*, *Arthritis & Rheumatism*, 1994, 37, 1664-1671), or into knee joints (Sack U., *et al.*, *J. Autoimmunity*, 1995, 9, 51-58). The implants grow with arthritis-like characteristics, including formation of pannus tissue of high cellular density, bone and cartilage erosion, development of multinuclear giant cells, and invasion of cartilage by synovial fibroblasts.

Indirect Method: Synoviocytes are transfected *in vitro* with the therapeutic gene and transplanted back in rabbits. Arthritis is induced in these rabbits by injecting IL-1 and expression of the therapeutic gene following activation is assessed. Activation-induced expression of the chimeric gene induces apoptosis in transplanted cells.

Direct Method: Intra-articular injection of the chimeric genes. Any of the gene delivery methods described above, including naked plasmid DNA,

cationic liposome-mediated delivery can be used. For use of viral vector-based delivery, chimeric genes are cloned in suitable vectors. The vectors are then modified by deleting eukaryotic promoter present in these vectors. Intra-articular injection of the therapeutic genes inserted in appropriate vectors can then be done
5 to assess therapeutic as well as prophylactic efficacy.

EXAMPLE 3

Selection of TNF- α Non-Producer Somatic Cell Variants

Cells (THP-1, Jurkat) are stably transfected *in vitro* with TNFp-AIG chimeric gene. After several cycles of stimulation, which induces apoptosis in the
10 cells expressing the TNFp-AIG gene, surviving cells are then collected. A cDNA library from these cells is constructed, which is used for functional cloning (Legerski R and Peterson C., *Nature*, 1992, 359, 70-73; Jaattela M., *et al.*, *Oncogene*, 1995, 10, 2297-2305).

EXAMPLE 4

Identification and Characterization of Dominant Negative (DN) Genes

THP-1 and Jurkat cells stably transfected with TNFp-AIG are subjected to repeated cycles of stimulation to activate expression of TNFp-AIG. The cells, which do not express negative regulatory genes, undergo apoptosis, whereas those
20 expressing dominant negative genes survive. In these surviving cells DN gene products act in-trans with the TNF α promoter, thereby inhibiting its activations to transcribe AIG, ultimately resulting in survival phenotype. cDNA library is constructed using polyadenylated mRNA from these cells. The DN genes which rescue TNFp-AIG-transfected THP-1 or Jurkat cells from apoptosis are identified by functional cloning as described for other genes (Legerski R. and Peterson C.,
25 *Nature*, 1992, 359, 70-73; Jaattela M., *et al.*, *Oncogene*, 1995, 10, 2297-2305).

The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

The papers cited herein are expressly incorporated in their entireties by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(A) TELEPHONE NUMBER: 203-798-4865
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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1178
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: DNA
- (ix) FEATURE:
 - (A) NAME/KEY: reference human TNF α promoter
- (x) PUBLICATION INFORMATION:
 - (A) AUTHORS: Takashiba, S., et al.
 - (C) JOURNAL: Gene
 - (D) VOLUME: 131
 - (e) PAGES: 307-308

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGGGAAGCAA	AGGAGAAGCT	GAGAAGATGA	AGGAAAAGTC	AGGGTCTGGA	50
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TGAGGAATGG	GTTACAGGAG	ACCTCTGGGG	AGATGTGACC	ACAGCAATGG	150
GTAGGAGAAT	GTCCAGGGCT	ATGGAAGTCG	AGTATCGGGG	ACCCCCCTT	200
AACGAAGACA	GGGCCATGTA	GAGGGCCCCA	GGGAGTGAAA	GAGCCTCCAG	250
GACCTCCAGG	TATGGAATAC	AGGGGACGTT	TAAGAAGATA	TGGCCACACA	300
CTGGGGCCCT	GAGAAGTGAG	AGCTTCATGA	AAAAAATCAG	GGACCCCA	350
GTTCTTGGA	AGCCAAGACT	GAAACCAGCA	TTATGAGTCT	CCGGGTCAGA	400
ATGAAAGAAG	AAGGCCTGCC	CCAGTGGTCT	GTGAATTCCC	GGGGGTGATT	450
TCACTCCCCG	GGCTGTCCCA	GGCTTGTCCC	TGCTACCCCC	ACCCAGCCTT	500
TCCTGAGGCC	TCAAGCTGCC	ACCAAGCCCC	CAGCTCCTTC	TCCCCGCAGA	550
CCCAAACACA	GGCCTCAGGA	CTCAACACAG	CTTTTCCCTC	CAACCCCGTT	600
TTCTCTCCCT	CAAGGACTCA	GCTTTCTGAA	GCCCCTCCCA	GTTCTAGTTC	650
TATCTTTTTC	CTGCATCCTG	TCTGGAAGTT	AGAAGGAAAC	AGACCACAGA	700
CCTGGTCCCC	AAAAGAAATG	GAGGCAATAG	GTTTTGAGGG	GCATGGGGAC	750
GGGGTTCAGC	CTCCAGGGTC	CTACACACAA	ATCAGTCAGT	GGCCCAGAAG	800
ACCCCCCTCG	GAATCGGAGC	AGGGAGGATG	GGGAGTGTGA	GGGGTATCCT	850
TGATGCTTGT	GTGTCCCCAA	CTTTCCAAAT	NCCCGCCCCC	GCGATGGAGA	900
AGAAACCGAG	ACAGAAGGTG	CAGGGCCAC	TACCGCTTCC	TCCAGATGAG	950
CTTATGGGTT	TCTCCACCAA	GGAAGTTTTC	CGCTGGTTGA	ATGATTCTTT	1000
CCCCGCCCTC	CTCTCGCCCC	AGGGACATAT	AAAGGCAGTT	GTTGGCACAC	1050
CCAGCCAGCA	GACGCTCCCT	CAGCAAGGAT	AGCAGAGGAC	CAGCTAAGAG	1100
GGAGAGAAGC	AACTGCAGAC	CCCCCCTGAA	AACAACCCTC	AGACGCCACA	1150
TCCCCTGACA	AGCTGCCAGG	CAGGTTCT			1178

(3) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1096
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
 - (A) DESCRIPTION: DNA
- (ix) FEATURE:
 - (A) NAME/KEY: human TNF α promoter gene

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Takashiba, S., et al.
 (C) JOURNAL: Gene
 (D) VOLUME: 131
 (e) PAGES: 307-308

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GAGGCCGCCA	GA	CTGCTGCA	GG	GGAAGCAA	AG	GAGAAGCT	GAG	AAGATGA	50
AGGAAAAGTC	AG	GGTCTGGA	GG	GGCGGGGG	TC	AGGGAGCT	CCT	GGGAGAT	100
ATGGCCACAT	GT	AGCGGCTC	TG	AGGAATGG	GT	TACAGGAG	AC	CTCTGGGG	150
AGATGTGACC	AC	AGCAATGG	GT	AGGAGAAT	GT	CCAGGGCT	AT	GGAAAGTCG	200
AGTATGGGGA	CCCC	CCCTTA	AC	GAAGACAG	GG	CCATGTAG	AG	GGGCCCCAG	250
GGAGTGAAAG	AG	CCTCCAGG	AC	CTCCAGGT	AT	GGAATACA	GG	GGACGTTT	300
AAGAAGATAT	GG	CCACACAC	TG	GGGGCCCTG	AG	AAGTGAGA	GCT	TCATGAA	350
AAAAATCAGG	GA	CCCCAGAG	TT	CCTTGGA	GC	CAAGACTG	AA	ACCAGCAT	400
TATGAGTCTC	CG	GGTCAGAA	TG	AAAGAAGA	AG	GCCTGCCC	CA	GTGGGGTC	450
TGTGAATTCC	CG	GGGGTGAT	TT	CACTCCCC	GG	GGCTGTCC	CA	GGCTTGTC	500
CCTGCTACCC	CC	ACCCAGCC	TT	TCTGAGG	CT	CAAGCCT	GC	CAACAAGC	550
CCCCAGCTCC	TT	CTCCCCGC	AG	GGACCCAA	AC	ACAGGCCT	CA	GGACTCAA	600
CACAGCTTTT	CC	CTCCAACC	CG	TTTTTCTC	TC	CCTCAAGG	AC	TCAGCTTT	650
CTGAAGCCCC	TC	CCAGTTCT	AG	TTCTATCT	TT	TTCTTGCA	TC	CTGTCTGG	700
AAGTTAGAAG	GA	AACAGACC	AC	AGACCTGG	TC	CCCCAAAAG	AA	ATGGAGGC	750
AATAGGTTTT	GAG	GGGCATG	GG	GACGGGGT	TC	AGCCTCCA	GG	GCCTACA	800
CACAAATCAG	TC	AGTGCCCC	AG	AAGACCCC	CT	CGGAATC	GG	AGCAGGGA	850
GGATGGGGAG	TG	TGAGGGGT	AT	CCTTGATG	CT	TGTGTGTC	CC	CAACTTTC	900
CAAATCCCCG	CCCC	CGCGAT	GG	AGAAGAAA	CC	GAGACAGA	AG	GTGCAGGG	950
CCCACTACCG	CT	TCTCCAG	AT	GAGCTCAT	GG	TTTCTCC	AC	CAAGGAAG	1000
TTTTCCGCTG	GT	TGAATGAT	TC	TTTCCCCG	CC	CTCCTCTC	GC	CCCAGGGA	1050
CATATAAAGG	CA	GTTGTTGG	CA	CACCCAGC	CA	GCAGACGC	TC	CCTC	1096

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: native minimal TNF α promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCGCTTCCTC	CA	GATGAGCT	CA	TGGGTTTC	TC	CAACAAGG	AA	GTTTTCCG	50
CTGGTTGAAT	GA	TTCTTTCC	CC	GCCCTCCT	CT	CGCCCCAG	GG	ACATATAA	100
AGGCAGTTGT	AT	GGCACACC	CG	CCAGCAGA	CG	CTCCCTC			139

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 904
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp120 AIG.1
- (D) OTHER INFORMATION: residues 1 to 139 comprise the promoter sequence; residues 140 to 151, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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CCGCTTCCTC CAGATGAGCT CATGGGTTTC TCCACCAAGG AAGTTTTCGG      50
CTGGTTGAAT GATTCTTTCC CCGCCCTCCT CTCGCCCCAG GGACATATAA     100
AGGCAGTTGT TGGCACACCC AGCCAGCAGA CGCTCCCTCA GCAGATCCAC      150
CATGTC TGGA ATATCCCTGG ACAACAGTTA TAAAATGGAT TATCCTGAGA      200
TGGGTTTATG TATAATAATT AATAATAAGA ATTTTCATAA AAGCACTGGA      250
ATGACATCTC GGTCTGGTAC AGATGTCGAT GCAGCAAACC TCAGGGAAAC      300
ATTCAGAAAC TTGAAATATG AAGTCAGGAA TAAAATGAT CTTACACGTG      350
AAGAAATTGT GGAATTGATG CGTGATGTTT CTAAAGAAGA TCACAGCAAA      400
AGGAGCAGTT TTGTTTGTGT GCTTCTGAGC CATGGTGAAG AAGGAATAAT      450
TTTTGGAACA AATGGACCTG TTGACCTGAA AAAAATAACA AACTTTTTTCA      500
GAGGGGATCG TTGTAGAAGT CTAAGTGGAA AACCCAACT TTTTATTATT      550
CAGGCCTGCC GTGGTACAGA ACTGGACTGT GGCATTGAGA CAGACAGTGG      600
TGTTGATGAT GACATGGCGT GTCATAAAAT ACCAGTGGAG GCCGACTTCT      650
TGTATGCATA CTCCACAGCA CTTGGTTATT ATTCTTGGCG AAATTCAAAG      700
GATGGCTCCT GGTTTCATCCA GTCGCTTTGT GCCATGCTGA AACAGTATGC      750
CGACAAGCTT GAATTTATGC ACATTCTTAC CCGGGCTAAC CGAAAGGTGG      800
CAACAGAATT TGAGTCCTTT TCCTTTGACG CTACTTTTCA TGCAAAGAAA      850
CAGATTCCAT GTATTGTTTC CATGCTCACA AAAGAACTCT ATTTTATCA      900
CTAA                                     904

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(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1490
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp706 AIG.1
- (D) OTHER INFORMATION: residues 1 to 724 comprise the

promoter sequence; residues 725 to 736, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TCCTTGGAAG	CCAAGACTGA	AACCAGCATT	ATGAGTCTCC	GGGTCAGAAT	50
GAAAGAAGAA	GGCCTGCCCC	AGTGGGGTCT	GTGAATTCCC	GGGGGTGATT	100
TCACTCCCCG	GGGCTGTCCC	AGGCTTGTCC	CTGCTACCCC	CACCCAGCCT	150
TTCCTGAGGC	TCAAGCCTGC	CACCAAGCCC	CCAGCTCCTT	CTCCCCGCAG	200
GGACCCAAAC	ACAGGCCTCA	GGACTCAACA	CAGCTTTTCC	CTCCAACCCC	250
GTTTTCTCTC	CCTCAAGGAC	TCAGCTTTCT	GAAGCCCCTC	CCAGTTCTAG	300
TTCTATCTTT	TTCTTGCATC	CTGTCTGGAA	GTTAGAAGGA	AACAGACCAC	350
AGACCTGGTC	CCCAAAGAA	ATGGAGGCAA	TAGGTTTTGA	GGGGCATGGG	400
GACGGGGTTC	AGCCTCCAGG	GTCCTACACA	CAAATCAGTC	AGTGGCCCAG	450
AAGACCCCCC	TCGGAATCGG	AGCAGGGAGG	ATGGGGAGTG	TGAGGGGTAT	500
CCTTGATGCT	TGTGTGTCCC	CAACTTTCCA	AATCCCCGCC	CCCGCGATGG	550
AGAAGAAACC	GAGACAGAAG	GTGCAGGGCC	CACTACCGCT	TCCTCCAGAT	600
GAGCTCATGG	GTTTCTCCAC	CAAGGAAGTT	TTCCGCTGGT	TGAATGATTC	650
TTTCCCCGCC	CTCCTCTCGC	CCCAGGGACA	TATAAAGGCA	GTTGTGCGCA	700
CACCCAGCCA	GCAGACGCTC	CCTCAGCAGA	TCCACCATGT	CTGGAATATC	750
CCTGGACAAC	AGTTATAAAA	TGGATTATCC	TGAGATGGGT	TTATGTATAA	800
TAATTAATAA	TAAGAATTTT	CATAAAAGCA	CTGGAATGAC	ATCTCGGTCT	850
GGTACAGATG	TCGATGCAGC	AAACCTCAGG	GAAACATTCA	GAAACTTGAA	900
ATATGAAGTC	AGGAATAAAA	ATGATCTTAC	ACGTGAAGAA	ATTGTGGAAT	950
TGATGCGTGA	TGTTTCTAAA	GAAGATCACA	GCAAAAGGAG	CAGTTTGTGT	1000
TGTGTGCTTC	TGAGCCATGG	TGAAGAAGGA	ATAATTTTGT	GAACAAATGG	1050
ACCTGTTGAC	CTGAAAAAAA	TAACAACTT	TTTCAGAGGG	GATCGTTGTA	1100
GAAGTCTAAC	TGGAAAACCC	AAACTTTTCA	TTATTCAGGC	CTGCCGTGGT	1150
ACAGAACTGG	ACTGTGGCAT	TGAGACAGAC	AGTGGTGTTG	ATGATGACAT	1200
GGCGTGTGAT	AAAATACCAG	TGGAGGCCGA	CTTCTTGAT	GCATACTCCA	1250
CAGCACCTGG	TTATTATTCT	TGGCGAAATT	CAAAGGATGG	CTCCTGGTTC	1300
ATCCAGTCGC	TTTGTGCCAT	TGCTGAAACA	GTATGCCGAC	AAGCTTGAAT	1350
TTATGCACAT	TCTTACCCGG	GCTAACCGAA	AGGTGGCAAC	AGAATTTGAG	1400
TCCTTTTCCT	TTGACGCTAC	TTTTCATGCA	AAGAAACAGA	TTCCATGTAT	1450
TGTTTCCATG	CTCACAAAAG	AACTCTATTT	TTATCACTAA		1490

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1789
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp1005 AIG.1
- (D) OTHER INFORMATION: residues 1 to 1023 comprise the promoter sequence; residues 1024 to 1036, the linker sequence, and the remaining residues comprise the AIG.1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGCGGGGGTTC	AGGGAGCTCC	TGGGAGATAT	GGCCACATGT	AGCGGCTCTG	50
AGGAATGGGT	TACAGGAGAC	CTCTGGGGAG	ATGTGACCAC	AGCAATGGGT	100
AGGAGAATGT	CCAGGGCTAT	GGAAGTCGAG	TATGGGGACC	CCCCCTTAAC	150
GAAGACAGGG	CCATGTAGAG	GGCCCCAGGG	AGTGAAAGAG	CCTCCAGGAC	200
CTCCAGGTAT	GGAATACAGG	GGACGTTTAA	GAAGATATGG	CCACACACTG	250
GGGCCCTGAG	AAGTGAGAGC	TTCATGAAAA	AAATCAGGGA	CCCCAGAGTT	300
CCTTGGAAGC	CAAGACTGAA	ACCAGCATTA	TGAGTCTCCG	GGTCAGAATG	350
AAAGAAGAAG	GCCTGCCCCA	GTGGGGTCTG	TGAATTCCCG	GGGGTGATTT	400
CACTCCCCGG	GGCTGTCCCA	GGCTTGTCCT	TGCTACCCCC	ACCCAGCCTT	450
TCCTGAGGCC	TCAAGCCTGC	CACCAAGCCC	CCAGCTCCTT	CTCCCCGCAG	500
GGACCCAAAC	ACAGGCCTCA	GGACTCAACA	CAGCTTTTCC	CTCCAACCCC	550
GTTTTCTCTC	CCTCAAGGAC	TCAGCTTTCT	GAAGCCCCTC	CCAGTTCTAG	600
TTCTATCTTT	TTCTGTCATC	CTGTCTGGAA	GTTAGAAGGA	AACAGACCAC	650
AGACCTGGTC	CCCAAAAGAA	ATGGAGGCAA	TAGGTTTTGA	GGGGCATGGG	700
GACGGGGTTC	AGCCTCCAGG	GTCCTACACA	CAAATCAGTC	AGTGGCCCAG	750
AAGACCCCCC	TCGGAATCGG	AGCAGGGAGG	ATGGGGAGTG	TGAGGGGTAT	800
CCTTGATGCT	TGTGTGTCCC	CAACTTTCCA	AATCCCCGCC	CCCGCGATGG	850
AGAAGAAACC	GAGACAGAAG	GTGCAGGGCC	CACTACCGCT	TCCTCCAGAT	900
GAGCTCATGG	GTTTCTCCAC	CAAGGAAGTT	TTCCGCTGGT	TGAATGATTC	950
TTTCCCCGCC	CTCCTCTCGC	CCCAGGGACA	TATAAAGGCA	GTTGTTGGCA	1000
CACCCAGCCA	GCAGACGCTC	CCTCAGCAGA	TCCACCATGT	CTGGAATATC	1050
CCTGGACAAC	AGTTATAAAA	TGGATTATCC	TGAGATGGGT	TTATGTATAA	1100
TAATTAATAA	TAAGAATTTT	CATAAAAGCA	CTGGAATGAC	ATCTCGGTCT	1150
GGTACAGATG	TCGATGCAGC	AAACCTCAGG	GAAACATTCA	GAAACTTGAA	1200
ATATGAAGTC	AGGAATAAAA	ATGATCTTAC	ACGTGAAGAA	ATTGTGGAAT	1250
TGATGCGTGA	TGTTTCTAAA	GAAGATCACA	GCAAAAGGAG	CAGTTTTGTT	1300
TGTGTGCTTC	TGAGCCATGG	TGAAGAAGGA	ATAATTTTTG	GAACAAATGG	1350
ACCTGTTGAC	CTGAAAAAAA	TAACAAACTT	TTTCAGAGGG	GATCGTTGTA	1400
GAAGTCTAAC	TGGAAAACCC	AAACTTTTCA	TTATTCAGGC	CTGCCGTGGT	1450
ACAGAACTGG	ACTGTGGCAT	TGAGACAGAC	AGTGGTGTTG	ATGATGACAT	1500
GGCGTGTCAT	AAAATACCAT	TGGAGGCCGA	CTTCTTGAT	GCATACTCCA	1550
CAGCACCTGG	TTATTATTCT	TGGCGAAATT	CAAAGGATGG	CTCCTGGTTC	1600
ATCCAGTCGC	TTTGTGCCAT	GCTGAAACAG	TATGCCGACA	AGCTTGAATT	1650
TATGCACATT	CTTACCCGGG	CTAACCGAAA	GGTGGCAACA	GAATTTGAGT	1700
CCTTTTCCTT	TGACGCTACT	TTTCATGCAA	AGAAACAGAT	TCCATGTATT	1750
GTTTCCATGC	TCACAAAAGA	ACTCTATTTT	TATCACTAA		1789

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1008
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp120 AIG.2
- (D) OTHER INFORMATION: residues 1 to 138 comprise the

promoter sequence; residues 139 to 150, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCGCTTCCTC	CAGATGAGCT	CATGGGTTTC	TCCACCAAGG	AAGTTTTCCTG	50
CTGGTTGAAT	GATTCTTTCC	CCGCCCTCCT	CTCGCCCCAG	GGACATATAA	100
AGGCAGTTGT	TGGCACACCC	AGCCAGCAGA	GCTCCCTCAG	CAGATCCACC	150
ATGGCTGGAC	CACCTGAGTC	AGCAGAATCT	ACAGATGCCC	TCAAGCTTTG	200
TCCTCATGAA	GAATTCCTGA	GACTATGTAA	AGAAAGAGCT	GAAGAGATCT	250
ACCCAATAAA	GGAGAGAAAC	AACCGCACAC	GCCTGGCTCT	CATCATATGC	300
AATACAGAGT	TTGACCATCT	GCCTCCGAGG	AATGGAGCTG	ACTTTGACAT	350
CACAGGGATG	AAGGAGCTAC	TTGAGGGTCT	GGACTATAGT	GTAAGATGTAG	400
AAGAGAATCT	GACAGCCAGG	GATATGGAGT	CAGCGCTGAG	GGCATTGCT	450
ACCAGACCAG	AGCACAAGTC	CTCTGACAGC	ACATTCTTGG	TACTCATGTC	500
TCATGGCATC	CTGGAGGGAA	TCTGCGGAAC	TGTGCATGAT	GAGAAAAAAC	550
CAGATGTGCT	GCTTTATGAC	ACCATCTTCC	AGATATTCAA	CAACCGCAAC	600
TGCCTCAGTC	TGAAGGACAA	ACCCAAGGTC	ATCATTGTCC	AGGCCTGCAG	650
AGGTGCAAAC	CGTGGGGAAC	TGTGGGTCAG	AGACTCTCCA	GCATCCTTGG	700
AAGTGGCCTC	TTCACAGTCA	TCTGAGAACC	TGGAGGAAGA	TGCTGTTTAC	750
AAGACCCACG	TGGAGAAGGA	CTTCATTGCT	TTCTGCTCTT	CAACGCCACA	800
CAACGTGTCC	TGGAGAGACA	GCACAAATGGG	CTCTATCTTC	ATCACACAAC	850
TCATCACATG	CTTCCAGAAA	TATTCTTGGT	GCTGCCACCT	AGAGGAAGTA	900
TTTCGGAAGG	TACAGCAATC	ATTTGAAACT	CCAAGGGCCA	AAGCTCAAAAT	950
GCCCACCATA	GAACGACTGT	CCATGACAAG	ATATTTCTAC	CTCTTTCCTG	1000
GCAATTGA					1008

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1587
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp706 AIG.2
- (D) OTHER INFORMATION: residues 1 to 724 comprise the promoter sequence; residues 725 to 736, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TCCTTGGAAG	CCAAGACTGA	AACCAGCATT	ATGAGTCTCC	GGGTCAGAAT	50
GAAAGAAGAA	GGCCTGCCCC	AGTGGGGTCT	GTGAATTCCC	GGGGGTGATT	100
TCACTCCCCG	GGCTGTCC	AGGCTTGTC	CTGCTACCCC	CACCCAGCCT	150
TTCCTGAGGC	CTCAAGCCTG	CCACCAAGCC	CCCAGCTCCT	TCTCCCCGCA	200
GGGACCCAAA	CACAGGCCTC	AGGACTCAAC	ACAGCTTTTC	CCTCCAACCC	250
CGTTTTCTCT	CCCTCAAGGA	CTCAGCTTTC	TGAAGCCCCT	CCCAGTTCTA	300
GTTCTATCTT	TTTCTGTCAT	CCTGTCTGGA	AGTTAGAAGG	AAACAGACCA	350

CAGACCTGGT	CCCCAAAAGA	AATGGAGGCA	ATAGGTTTTG	AGGGGCATGG	400
GGACGGGGTT	CAGCCTCCAG	GGTCCTACAC	ACAAATCAGT	CAGTGGCCCA	450
AAGACCCCCC	TCGGAATCGG	AGCAGGGAGG	ATGGGGAGTG	TGAGGGGTAT	500
CCTTGATGCT	TGTGTGTCCC	CAACTTTCCA	AATCCCCGCC	CCCGCGATGG	550
AGAAGAAACC	GAGACAGAAG	GTGCAGGGCC	CACTACCGCT	TCCTCCAGAT	600
GAGCTCATGG	TTTTCTCCAC	CAAGGAAGTT	TTCCGCTGGT	TGAATGATTC	650
TTTCCCCGCC	CTCCTCTCGC	CCCAGGGACA	TATAAAGGCA	GTTGTTGGCA	700
CACCCAGCCA	GCAGACGCTC	CCTCAGCAGA	TCCACCATGG	CTGGACCACC	750
TGAGTCAGCA	GAATCTACAG	ATGCCCTCAA	GCTTTGTCCT	CATGAAGAAT	800
TCCTGAGACT	ATGTAAAGAA	AGAGCTGAAG	AGATCTACCC	AATAAAGGAG	850
AGAAACAACC	GCACACGCCCT	GGCTCTCATC	ATATGCAATA	CAGAGTTTGA	900
CCATCTGCCT	CCGAGGAATG	GAGCTGACTT	GACATCACAG	GATGAAGGAG	950
TACTTGAGGG	TCTGGACTAT	GTGTAGATGT	GAAGAGAATC	GACAGCCAGG	1000
ATATGGAGTC	AGCGCTGAGG	GCATTTGCTA	CCAGACCAGA	GCACAAGTCC	1050
TCTGACAGCA	CATTCTTGGT	ACTCATGTCT	CATGGCATCC	TGGAGGGAAT	1100
CTGCGGAACT	GTGCATGATG	AGAAAAAACC	AGATGTGCTG	CTTTATGACA	1150
CCATCTTCCA	GATATTCAAC	AACCGCAACT	GCCTCAGTCT	GAAGGACAAA	1200
CCCAAGGTCA	TCATTGTCCA	GGCCTGCAGA	GGTGCAAACC	GTGGGGAACCT	1250
GTGGGTCAGA	GACTCTCCAG	CATCCTTGGA	AGTGGCCTCT	TCACAGTCAT	1300
CTGAGAACCT	GGAGGAAGAT	GCTGTTTACA	AGACCCACGT	GGAGAAGGAC	1350
TTCATTGCTT	TCTGCTCTTC	AACGCCACAC	AACGTGTCCT	GGAGAGACAG	1400
CACAATGGGC	TCTATCTTCA	TCACACAACT	CATCACATGC	TTCCAGAAAT	1450
ATTCTTGGTG	CTGCCACCTA	GAGGAAGTAT	TTCGGAAGGT	ACAGCAATCA	1500
TTTGAAACTC	CAAGGGCCAA	AGCTCAAATG	CCCACCATAG	AACGACTGTC	1550
CATGACAAGA	TATTTCTACC	TCTTTCCTGG	CAATTGA		1587

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1894
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: chimeric gene TNFp1005 AIG.2
- (D) OTHER INFORMATION: residues 1 to 1024 comprise the promoter sequence; residues 1025 to 1036, the linker sequence, and the remaining residues comprise the AIG.2 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGCGGGGGTC	AGGGAGCTCC	TGGGAGATAT	GGCCACATGT	AGCGGCTCTG	50
AGGAATGGGT	TACAGGAGAC	CTCTGGGGAG	ATGTGACCAC	AGCAATGGGT	100
AGGAGAATGT	CCAGGGCTAT	GGAAGTCGAG	TATGGGGACC	CCCCCTTAAC	150
GAAGACAGGG	CCATGTAGAG	GGCCCCAGGG	AGTGAAAGAG	CCTCCAGGAC	200
CTCCAGGTAT	GGAATACAGG	GGACGTTTAA	GAAGATATGG	CCACACACTG	250
GGGCCCTGAG	AAGTGAGAGC	TTCATGAAAA	AAATCAGGGA	CCCCAGAGTT	300
CCTTGGAAGC	CAAGACTGAA	ACCAGCATTA	TGAGTCTCCG	GGTCAGAATG	350
AAAGAAGAAG	GCCTGCCCCA	GTGGGGTCTG	TGAATTCCCG	GGGGTGATTT	400

CACTCCCCGG	GGCTGTCCCA	GGCTTGTTCC	TGCTACCCCC	ACCCAGCCTT	450
TCCTGAGGCC	TCAAGCCTGC	CACCAAGCCC	CCAGCTCCTT	CTCCCCGCAG	500
GGACCCAAAC	ACAGGCCTCA	GGACTCAACA	CAGCTTTTCC	CTCCAACCCC	550
GTTTTCTCTC	CCTCAAGGAC	TCAGCTTTCT	GAAGCCCCTC	CCAGTTCTAG	600
TTCTATCTTT	TTCCTGCATC	CTGTCTGGAA	GTTAGAAGGA	AACAGACCAC	650
AGACCTGGTC	CCCAAAAGAA	ATGGAGGCAA	TAGGTTTTGA	GGGGCATGGG	700
GACGGGGTTC	AGCCTCCAGG	GTCCTACACA	CAAATCAGTC	AGTGGCCCAG	750
AAGACCCCCC	TCGGAATCGG	AGCAGGGAGG	ATGGGGAGTG	TGAGGGGTAT	800
CCTTGATGCT	TGTGTGTCCC	CAACTTTCCA	AATCCCCGCC	CCCGCGATGG	850
AGAAGAAACC	GAGACAGAAG	GTGCAGGGCC	CACTACCGCT	TCCTCCAGAT	900
GAGCTCATGG	GTTTCTCCAC	CAAGGAAGTT	TTCCGCTGGT	TGAATGATTC	950
TTTCCCCGCC	CTCCTCTCGC	CCCAGGGACA	TATAAAGGCA	GTTGTTGGCA	1000
CACCCAGCCA	GCAGACGCTC	CCTCAGCAGA	TCCACCATGG	CTGGACCACC	1050
TGAGTCAGCA	GAATCTACAG	ATGCCCTCAA	GCTTTGTCCT	CATGAAGAAT	1100
TCCTGAGACT	ATGTAAAGAA	AGAGCTGAAG	AGATCTACCC	AATAAAGGAG	1150
AGAAACAACC	GCACACGCCT	GGCTCTCATC	ATATGCAATA	CAGAGTTTGA	1200
CCATCTGCCT	CCGAGGAATG	GAGCTGACTT	TGACATCACA	GGGATGAAGG	1250
AGCTACTTGA	GGGTCTGGAC	TATAGTGTAG	ATGTAGAAGA	GAATCTGACA	1300
GCCAGGGATA	TGGAGTCAGC	GCTGAGGGCA	TTTGCTACCA	GACCAGAGCA	1350
CAAGTCCCTC	GACAGCACAT	TCTTGGTACT	CATGTCTCAT	GGCATCCTGG	1400
AGGGAATCTG	CGGAAGTGTG	CATGATGAGA	AAAAACCAGA	TGTGCTGCTT	1450
TATGACACCA	TCTTCCAGAT	ATTCAACAAC	CGCAACTGCC	TCAGTCTGAA	1500
GGACAAACCC	AAGGTCATCA	TTGTCCAGGC	CTGCAGAGGT	GCAAACCGTG	1550
GGGAAGTGTG	GGTCAGAGAC	TCTCCAGCAT	CCTTGGAAGT	GGCCTCTTCA	1600
CAGTCATCTG	AGAACCTGGA	GGAAGATGCT	GTTTACAAGA	CCCACGTGGA	1650
GAAGGACTTC	ATTGCTTTCT	GCTCTTCAAC	GCCACACAAC	GTGTCCTGGA	1700
GAGACAGCAC	AATGGGCTCT	ATCTTCATCA	CACAACTCAT	CACATGCTTC	1750
CAGAAATATT	CTTGGTGCTG	CCACCTAGAG	GAAGTATTTT	GGAAGGTACA	1800
GCAATCATTT	GAAACTCCAA	GGGCCAAAGC	TCAAATGCCC	ACCATAGAAC	1850
GACTGTCCAT	GACAAGATAT	TTCTACCTCT	TTCTTGCGAA	TTGA	1894

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: TNF α promoter enhancer region 1 (ER1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGGGCGGGGG	TCAGGGAGCT	CCTGGGAGAT	ATGGCCACAT	GTAGCGGCTC	50
TGAGGAATGG	GTTACAGGAG	ACCTCTGGGG	AGATGTGACC	ACAGCAATGG	100
GTAGGAGAAT	GTCCAGGGCT	ATG			123

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 190
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: TNF α promoter enhancer region 2 (ER2)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TCCTTGGAAG CCAAGACTGA AACCAGCATT ATGAGTCTCC GGGTCAGAAT	50
GAAAGAAGAA GGCCTGCCCC AGTGGGGTCT GTGAATTCCC GGGGGTGATT	100
TCACTCCCCG GGGCTGTCCC AGGCTTGTCC CTGCTACCCC CACCCAGCCT	150
TTCCTGAGGC CTCAAGCCTG CCACCAAGCC CCCAGCTCCT	190

(13) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 223
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: DNA

(ix) FEATURE:

- (A) NAME/KEY: multiple cloning sites

(D) OTHER INFORMATION: genetically engineered multiple cloning sites genetically engineered upstream of the minimal TNF α promoter in the -120pGL3 construct

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGTACCGAGC TCTTACGCGT GCTAGCCGCG GATATCTTAA GACGTCCTAG	50
GACTAGTCAG CTGCTCGAGC CGCTTCCTCC AGATGAGCTC ATGGGTTTCT	100
CCACCAAGGA AGTTTTCCGC TGGTTGAATG ATTCTTTCCC CGCCCTCCTC	150
TCGCCCCAGG GACATATAAA GGCAGTTGTT GGCACACCCA GCCAGCAGAC	200
GCTCCCTCAG CAGATCTAAG CTT	223

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 787
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: DNA

(ix) FEATURE:
(A) NAME/KEY: TNF α intranlated region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TCTAGAGGAG	GACGAACATC	CAACCTTCCC	AAACGCCTCC	CCTGCCCCAA	50
TCCCTTTATT	ACCCCTCCT	TCAGACACCC	TCAACCTCTT	CTGGCTCAAA	100
AAGAGAATTG	GGGGCTTAGG	GTCCGAACCC	AAGCTTAGAA	CTTTAAGCAA	150
CAAGACCACC	ACTTCGAAAC	CTGGGATTCA	GGAATGTGTG	GCCTGCACAG	200
TGAAGTGCTG	GCAACCACTA	AGAATTCAAA	CTGGGGCCTC	CAGAACTCAC	250
TGGGGCCTAC	AGCTTTGATC	CCTGACATCT	GGAATCTGGA	GACCAGGGAG	300
CCTTTGGTTC	TGGCCAGAAT	GCTGCAGGAC	TTGAGAAGAC	CTCACCTAGA	350
AATTGACACA	AGTGGACCTT	AGGCCTTCCT	CTCTCCAGAT	GTTTCCAGAC	400
TTCCTTGAGA	CACGGAGCCC	AGCCCTCCCC	ATGGAGCCAG	CTCCCTCTAT	450
TTATGTTTGC	ACTTGTGATT	ATTTATTATT	TATTTATTAT	TTATTTATTT	500
ACAGATGAAT	GTATTTATTT	GGGAGACCGG	GGTATCCTGG	GGGACCCAAT	550
GTAGGAGCTG	CCTTGGCTCA	GACATGTTTT	CCGTGAAAAC	GGAGCTGAAC	600
AATAGGCTGT	TCCCATGTAG	CCCCCTGGCC	TCTGTGCCTT	CTTTTGATTA	650
TGTTTTTTAA	AATATTTATC	TGATTAAGTT	GTCTAAACAA	TGCTGATTTG	700
GTGACCAACT	GTCACTCATT	GCTGAGCCTC	TGCTCCCCAG	GGGAGTTGTG	750
TCTGTAATCG	CCCTACTATT	CAGTGGCGAG	ATCTAGA		787

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CLAIMS

1. A chimeric gene comprising at least one TNF α promoter enhancer attached to a functional copy of a minimal TNF α promoter and further attached to at least one copy of an apoptosis-inducing gene, wherein the expression of the apoptosis-inducing gene is driven by the TNF α promoter.
2. A gene according to claim 1 wherein the attachment of the enhancer to the promoter and the promoter to the apoptosis-inducing gene is selected from the group consisting of direct attachment, distal attachment, proximal attachment, and combinations thereof.
3. A gene according to claim 1 comprising 2 or more copies of the TNF α promoter enhancer.
4. A gene according to claim 1 wherein the TNF α promoter enhancer is SEQ ID NO: 10 or SEQ ID NO: 11, or functional fragments or variants thereof.
5. A gene according to claim 1 wherein the TNF α promoter is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and functional fragments or variants thereof.
6. A gene according to claim 1 wherein the apoptosis-inducing gene is selected from the group consisting of caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, Granzyme A, Granzyme B, Fas ligand, and functional fragments, variants, and mixtures of any of these.

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7. A gene according to claim 6 wherein the apoptosis-inducing gene is selected from the group consisting of caspase 3, caspase 4, caspase 5, Granzyme B, and functional fragments, variants, and mixtures of any of these.
8. A gene according to claim 1 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof.
9. A gene according to claim 1 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof, wherein the 3'UTR of the TNF α gene is ligated downstream of the apoptosis-inducing gene.
10. A pharmaceutical composition comprising a gene according to claim 1.
11. A pharmaceutical composition comprising a gene according to claim 9.
12. A method for treating an inflammatory disorder in a patient comprising the step of inducing apoptosis in inflammatory cells or cells at a site of inflammation of the patient by introducing into the cells a chimeric gene according to claim 1.
13. A method according to claim 12 wherein the induction of apoptosis does not induce an inflammatory response in the patient.
14. A method according to claim 12 wherein the inflammatory cell is a TNF α producing cell.
15. A method according to claim 12 wherein the inflammatory disorder is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, Guillain-Barre syndrome, Crohn's disease, ulcerative colitis, psoriasis, graft versus

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host disease, lupus erythematosus, insulin-dependent diabetes mellitus, psoriatic
5 arthritis, sarcoidosis, hypersensitivity pneumonitis, ankylosing spondylitis, Reiter's
syndrome, and systemic sclerosis.

16. A method according to claim 15 wherein the inflammatory disorder is
rheumatoid arthritis.

17. A chimeric gene comprising 2 to 10 cassettes of a $\text{TNF}\alpha$ promoter enhancer
attached to at least one copy of a minimal $\text{TNF}\alpha$ promoter, and at least one copy
of an apoptosis-inducing gene selected from the group consisting of caspase 3,
caspase 4, caspase 5, Granzyme B and functional fragments, variants, and mix-
5 tures of any of these, wherein the expression of the apoptosis-inducing gene is
driven by the TNF promoter.

18. A pharmaceutical composition comprising a gene according to claim 17.

19. A method for inducing apoptosis in a $\text{TNF}\alpha$ -producing inflammatory cell by
introducing into the cell a chimeric gene according to claim 17.

20. A method for treating an inflammatory disorder in a patient comprising
inducing apoptosis in inflammatory cells or cells at the site of inflammation of the
patient by introducing into the cells a chimeric gene according to claim 17 without
inducing an inflammatory response in the patient.

21. A process for constructing a chimeric gene comprising at least one $\text{TNF}\alpha$
promoter enhancer attached to a functional copy of a minimal $\text{TNF}\alpha$ promoter and
further attached to at least one copy of an apoptosis-inducing gene, wherein the
expression of the apoptosis-inducing gene is driven by the $\text{TNF}\alpha$ promoter
5 comprising the steps of

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- (a) amplifying a TNF α promoter by a polymerase chain reaction using primers encompassing deletion constructs of the TNF α promoter;
- (b) cloning the PCR-amplified genes obtained in step (a) upstream of a reporter gene;
- 10 (c) testing the constructs obtained in step (b) for their constitutive and inducible expression in at least one TNF α -producing cell line;
- (d) selecting TNF α promoter responsible for inducible expression of the reporter in the cell line; and either
- (e) PCR-amplifying TNF α promoter regions that enhance expression of
15 the reporter to obtain an enhancer and ligating at least one copy of the enhancer upstream of the promoter; or
- (f) inserting at least one copy of a prodomain-deleted apoptosis-inducing gene downstream of the TNF α promoter by replacing the reporter gene with the apoptosis-inducing gene deletion constructs to obtain a chimeric gene or
- 20 (g) PCR-amplifying a TNF α -3'UTR and ligating downstream of the reporter gene, or any combination of these procedures.

22. A process according to claim 21 wherein 2 or more copies of the enhancer are inserted upstream of the promoter.

23. A process according to claim 21 wherein reporter gene is luciferase.

24. A process according to claim 21 wherein the enhancer comprises SEQ ID NO: 10 or SEQ ID NO: 11.

25. A process according to claim 21 wherein the prodomain-deleted apoptosis-inducing gene is selected from the group consisting of caspase 3, caspase 4, caspase 5, Granzyme B, and functional fragments and variants thereof.

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27. A process according to claim 21 producing a gene selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and functional fragments or variants thereof.

28. A process according to claim 21 wherein the cell lines for testing constructs are selected from the group consisting of T lymphoblastoid, myelomonocytic, monocytic, fibroblast, and cultured human synoviocytes.

29. A chimeric gene comprising:

(a) at least one promoter enhancer attached to a functional copy of a minimal promoter, provided that the promoter is a gene or combination of genes activated in inflammatory cells or in cells at a site of inflammation, and

5 (b) further attached to at least one copy of an apoptosis-inducing gene, wherein the expression of the apoptosis-inducing gene is driven by the promoter, and the promoter is selected from the group consisting of cytokines, interleukins and their receptors, cell adhesion molecules and their ligands, chemokines and their receptors, pro-inflammatory enzymes, and mixtures thereof.

31. A gene according to claim 29 wherein the promoter is selected from the group consisting of TNF β , IL-1 α , IL-1 β , IL-2, IL-6, IL-8, GM-CSF, interferon γ , functional fragments and variants thereof, and mixtures of any of these.

32. A gene according to claim 29 wherein the promoter is selected from the group consisting of selectins, integrins, ICAM-1, V-CAM, functional fragments and variants thereof, and mixtures of any of these.

33. A gene according to claim 29 wherein the promoter is selected from the group consisting of MIP-1 α , MIP-1 β , MCP1-4, RANTES, Mig, NAP2, IP10, Gro α - γ , functional fragments and variants thereof, and mixtures of any of these.

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34. A gene according to claim 29 wherein the promoter is selected from the group consisting of COX-2, iNOS, phospholipases, proteases, functional fragments and variants thereof, and mixtures of any of these.

35. A gene according to claim 29 wherein the attachment of the enhancer to the promoter and the promoter to the apoptosis-inducing gene is selected from the group consisting of direct attachment, distal attachment, proximal attachment, and combinations thereof.

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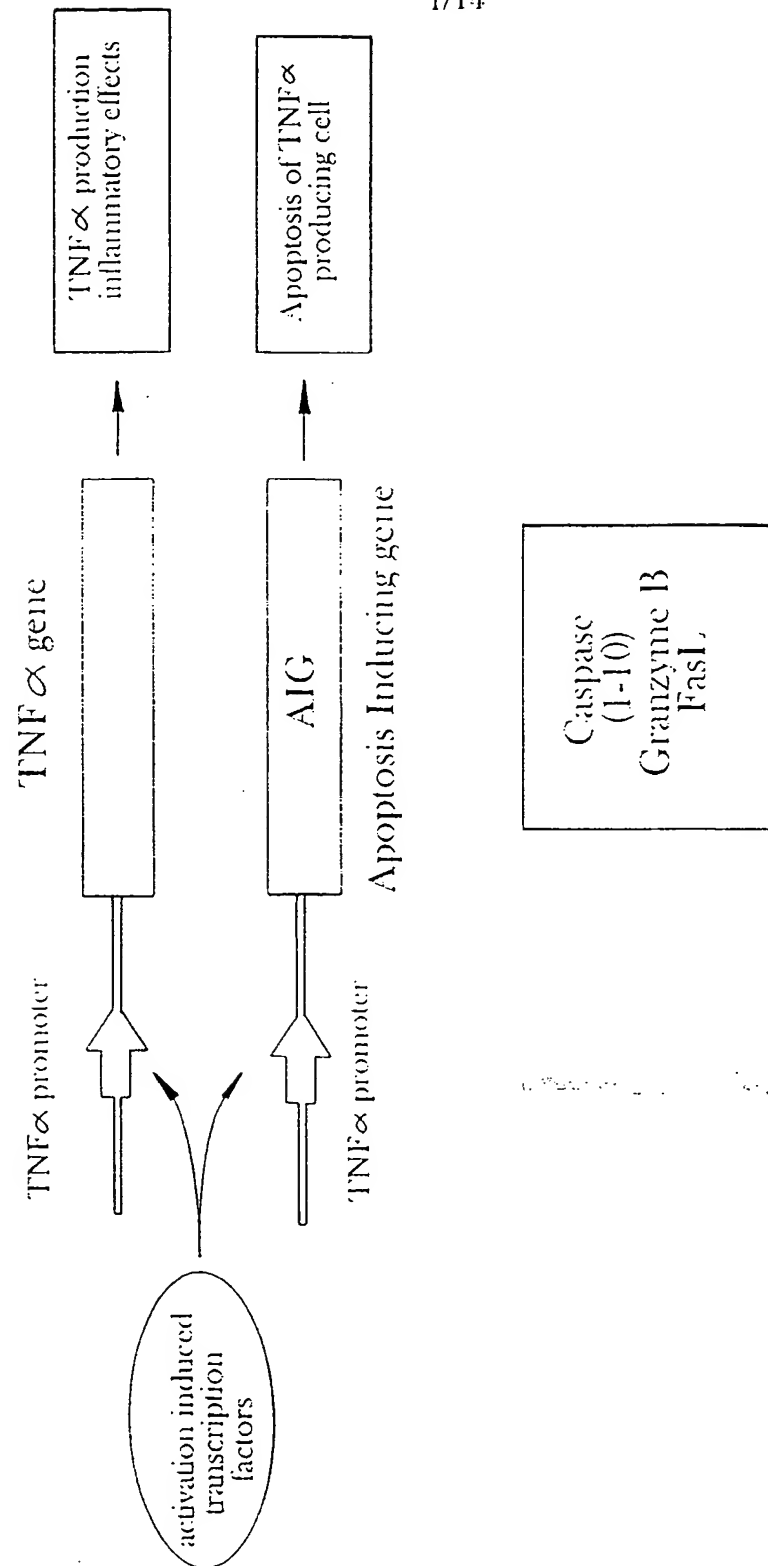


Fig. 1

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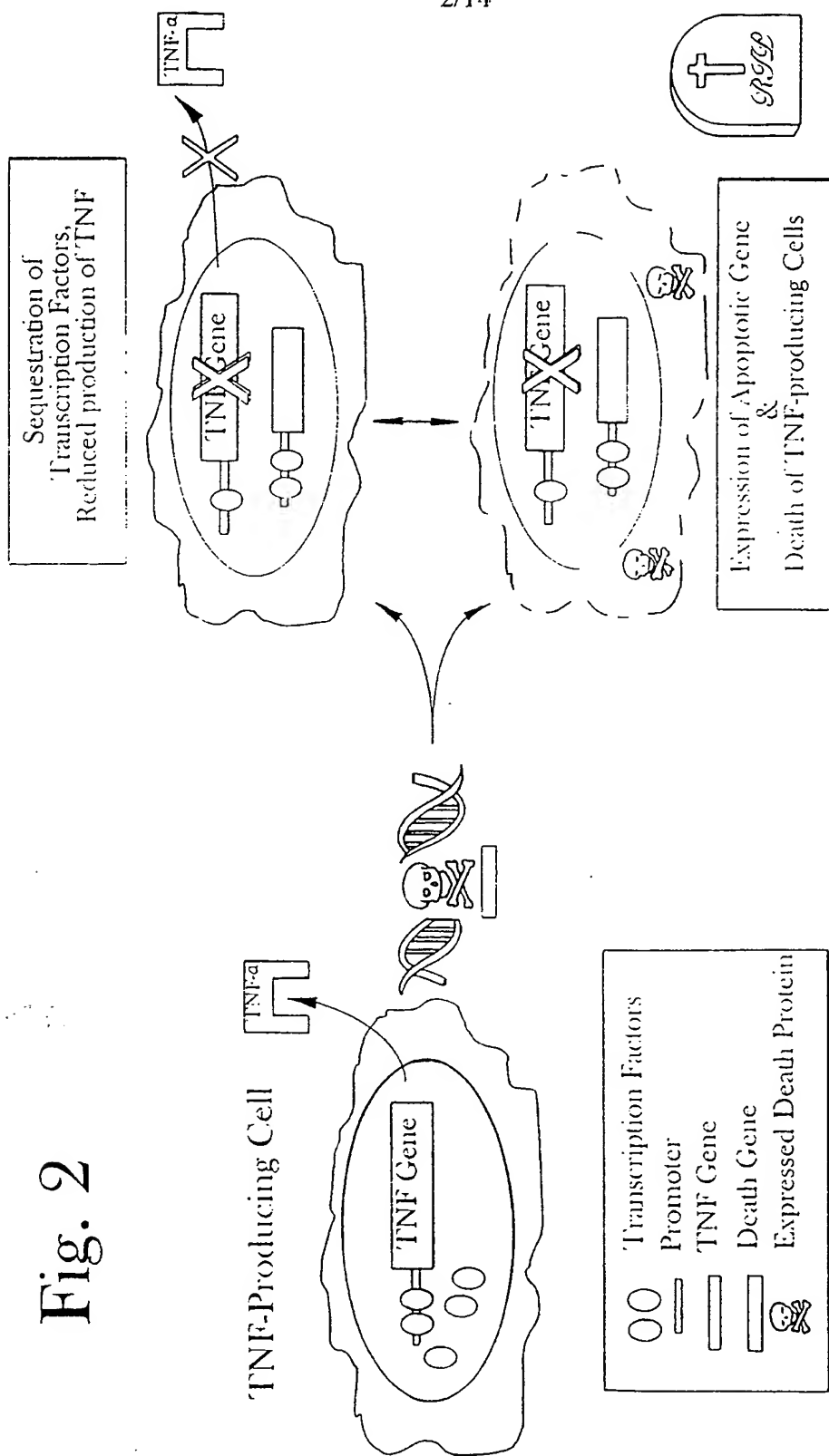


Fig. 2

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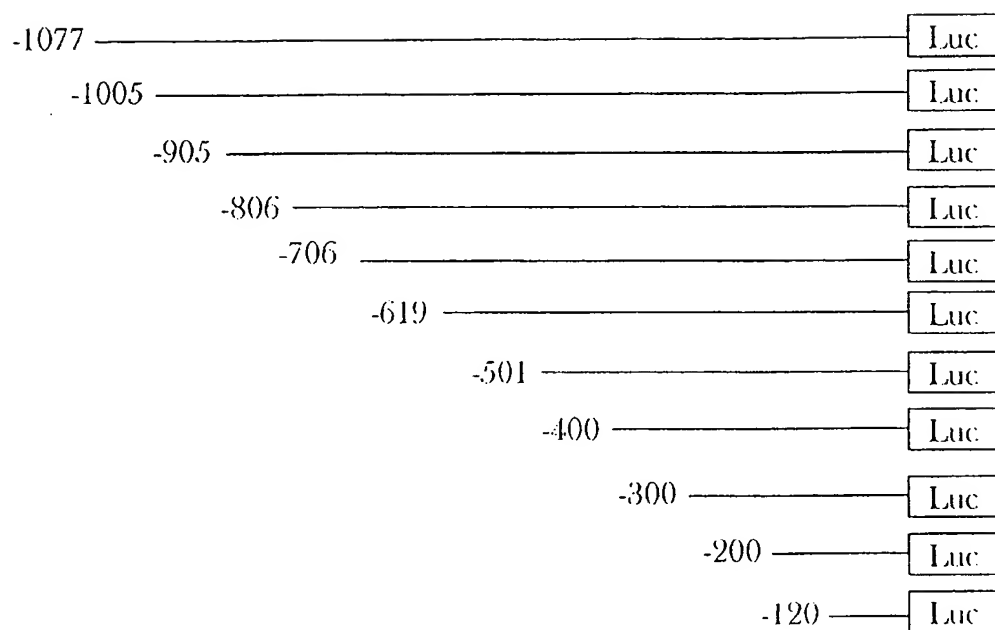


Fig. 3

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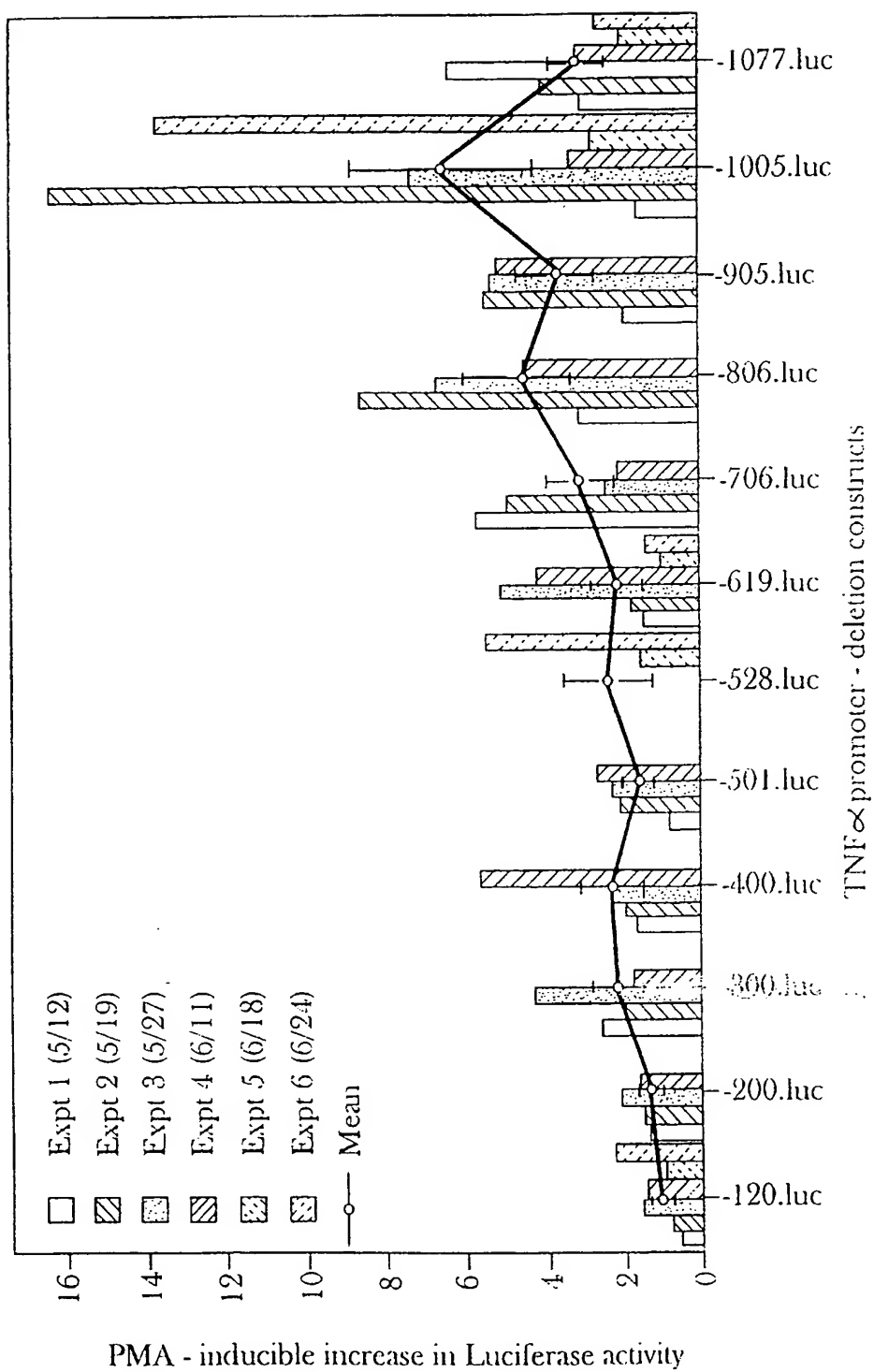


Fig. 4a

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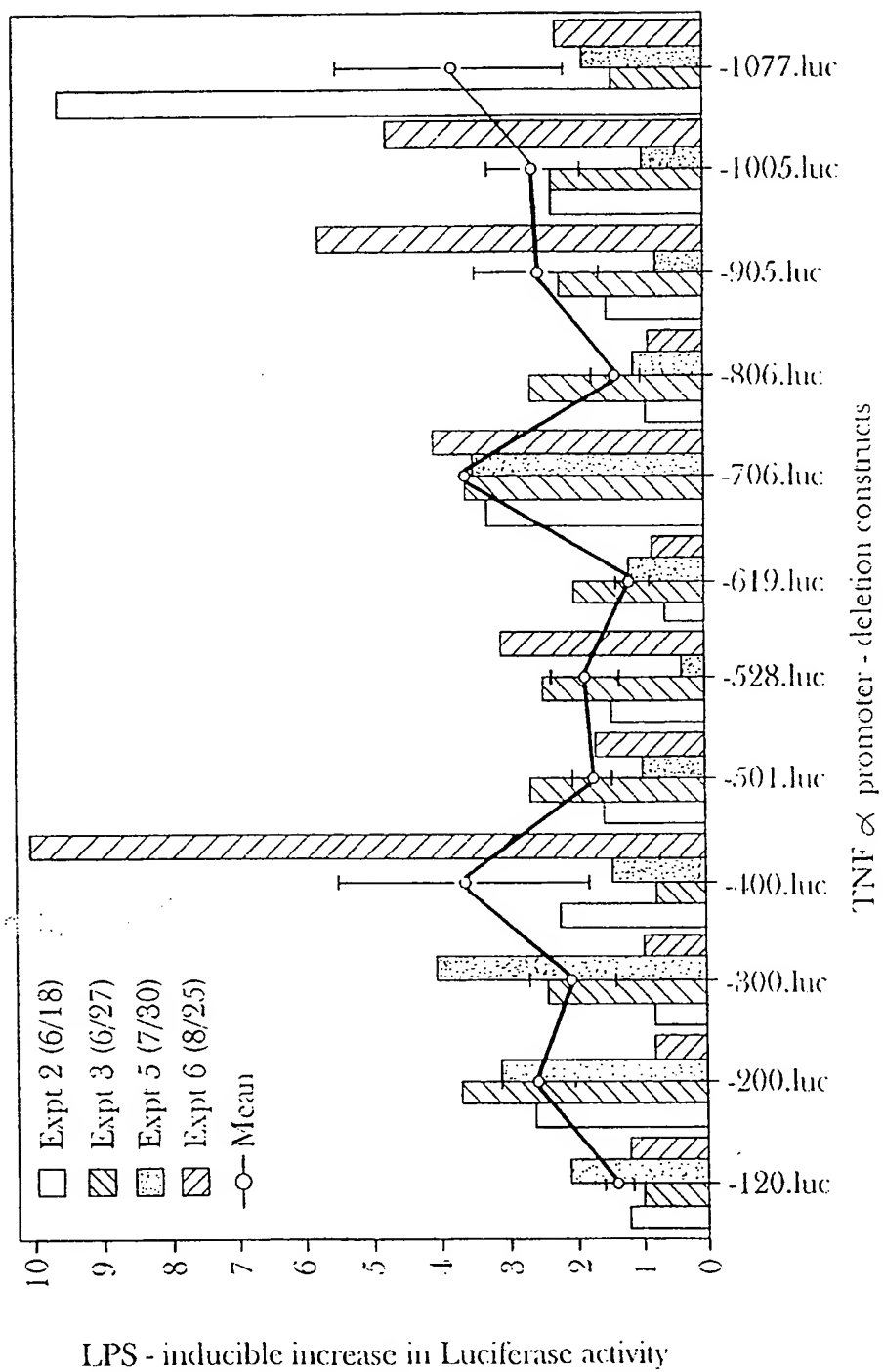


Fig. 4b

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pGL3 basic vector elements

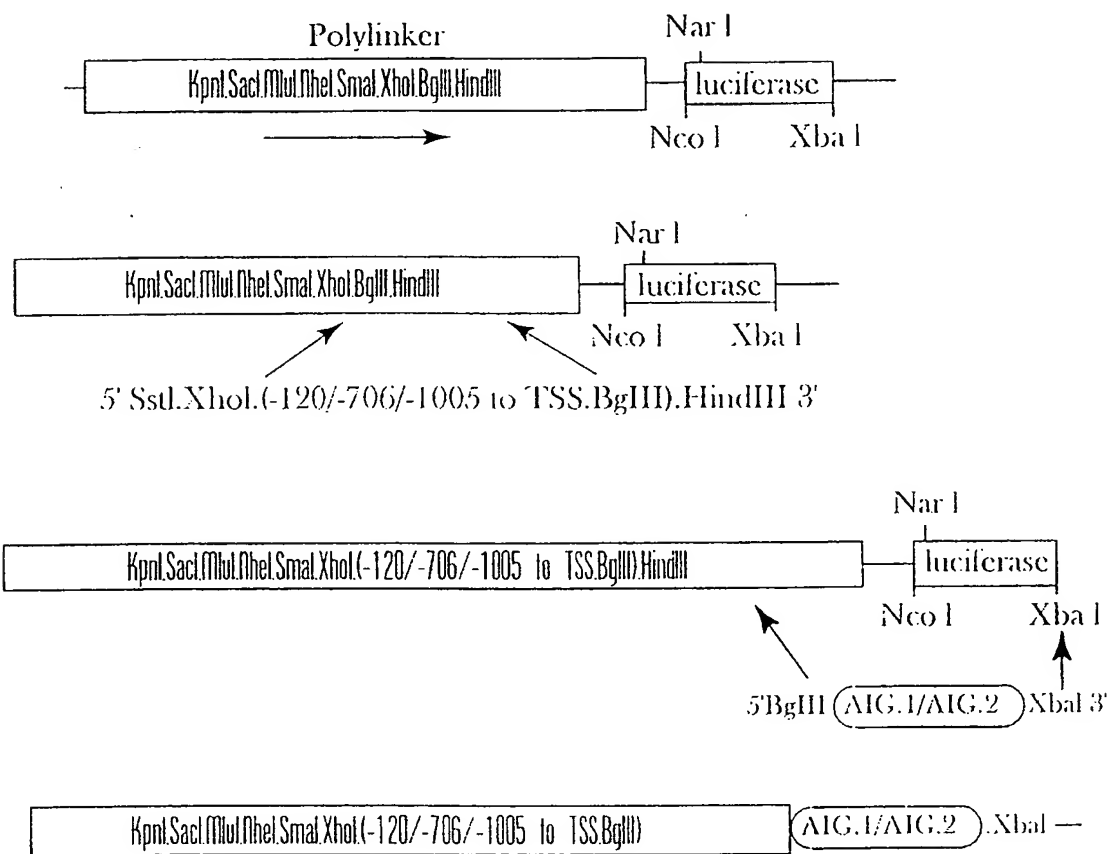


Fig. 5

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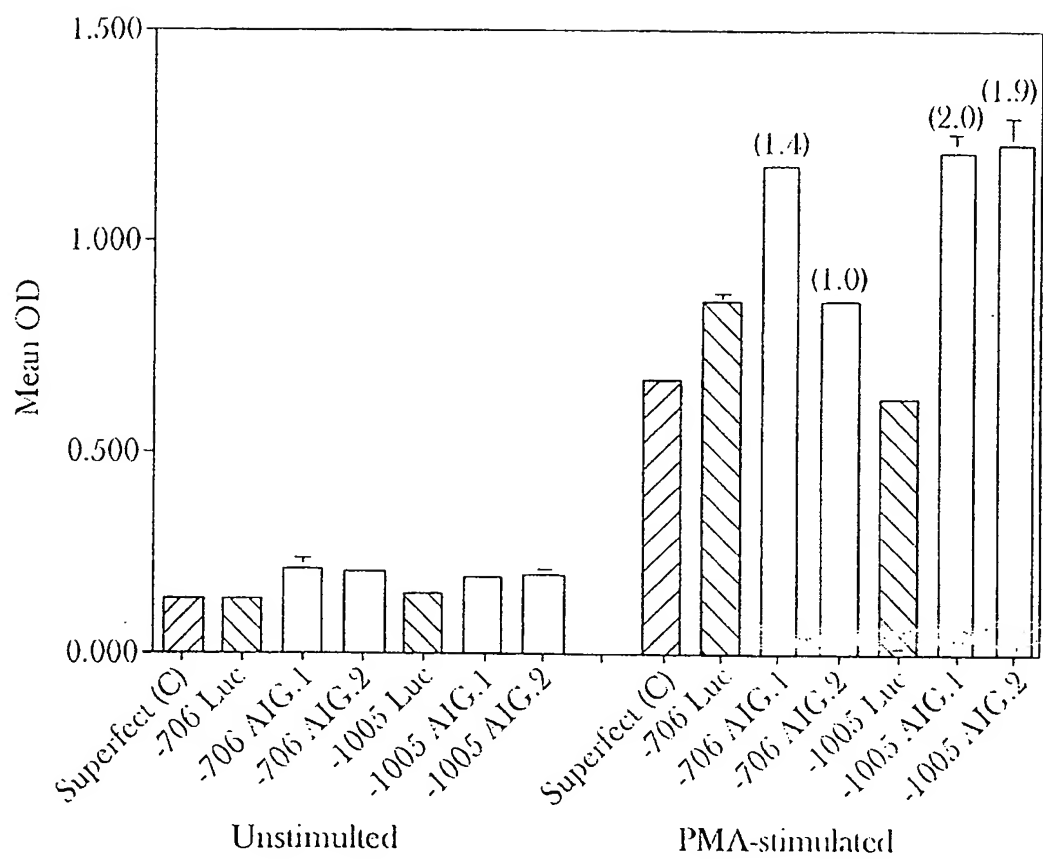


Fig. 6a

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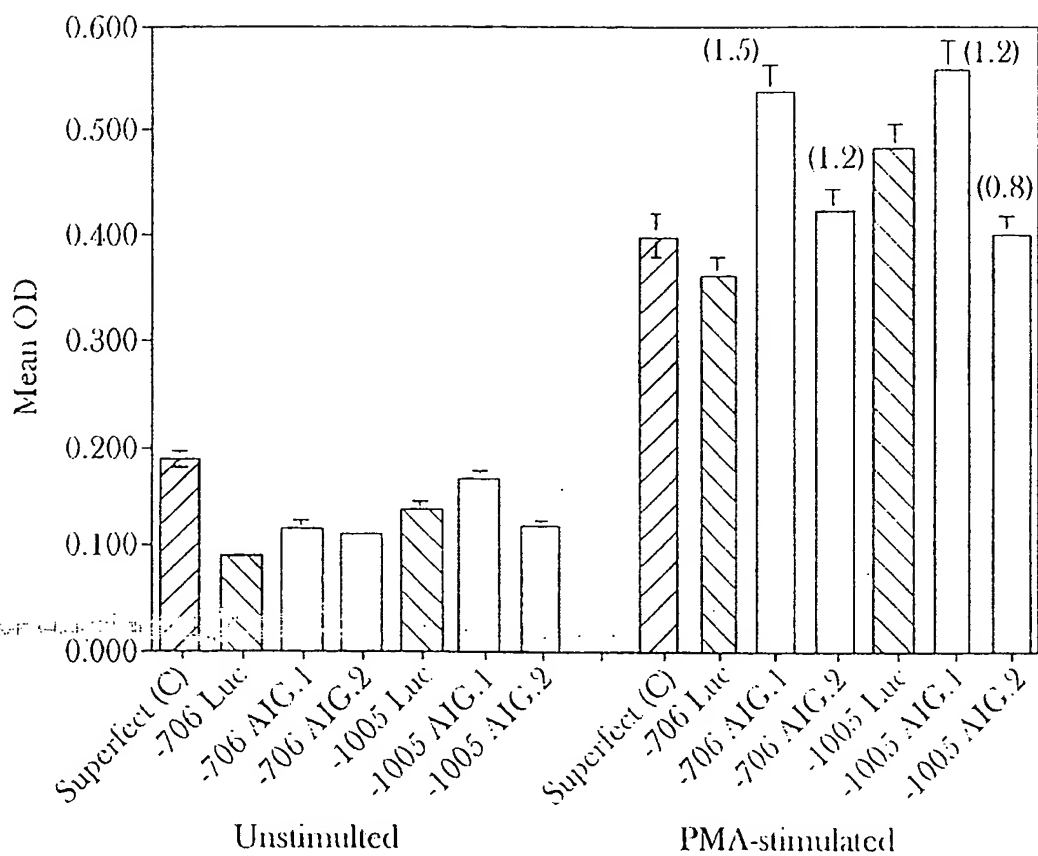


Fig. 6b

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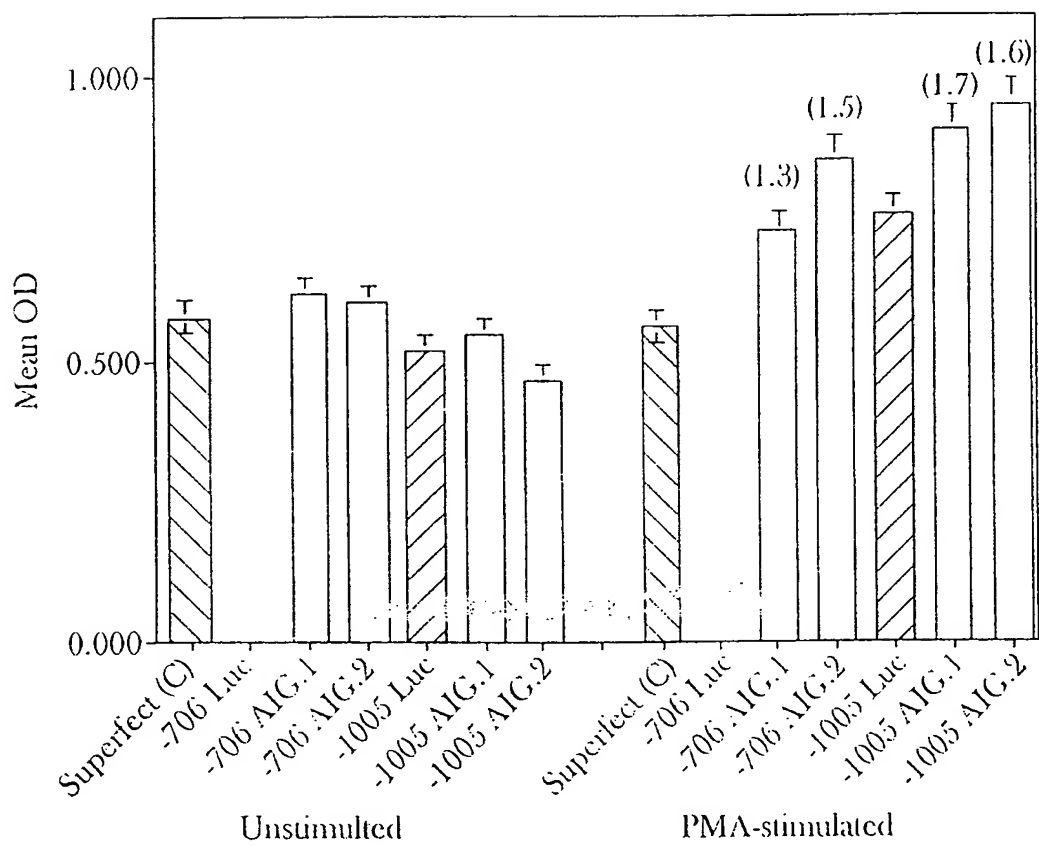


Fig. 6c

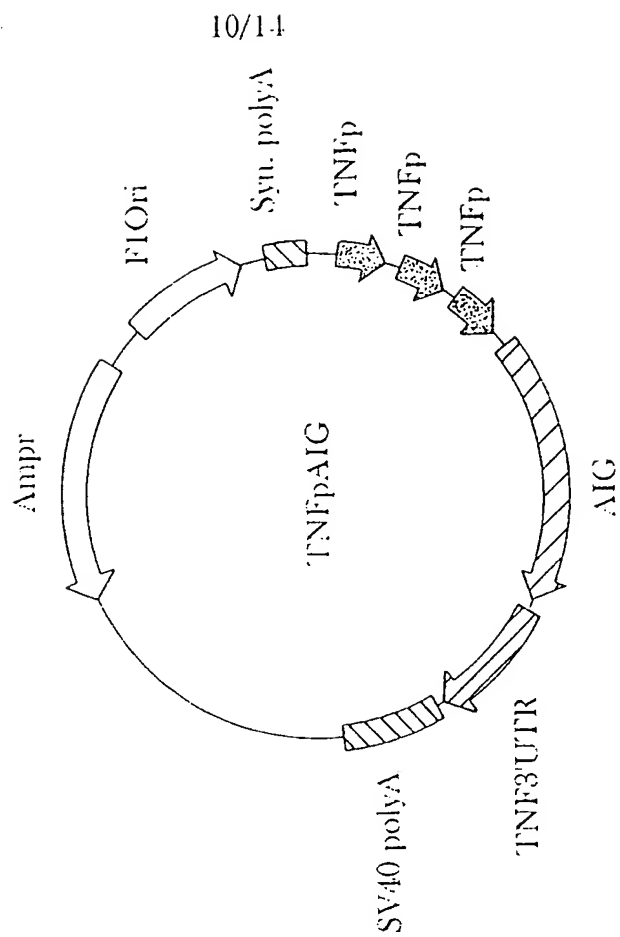


Fig. 7A

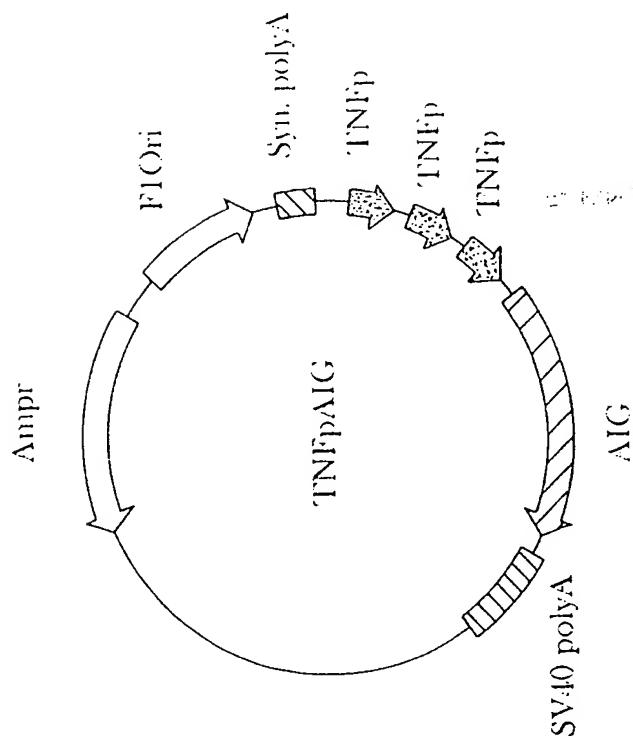


Fig. 7B

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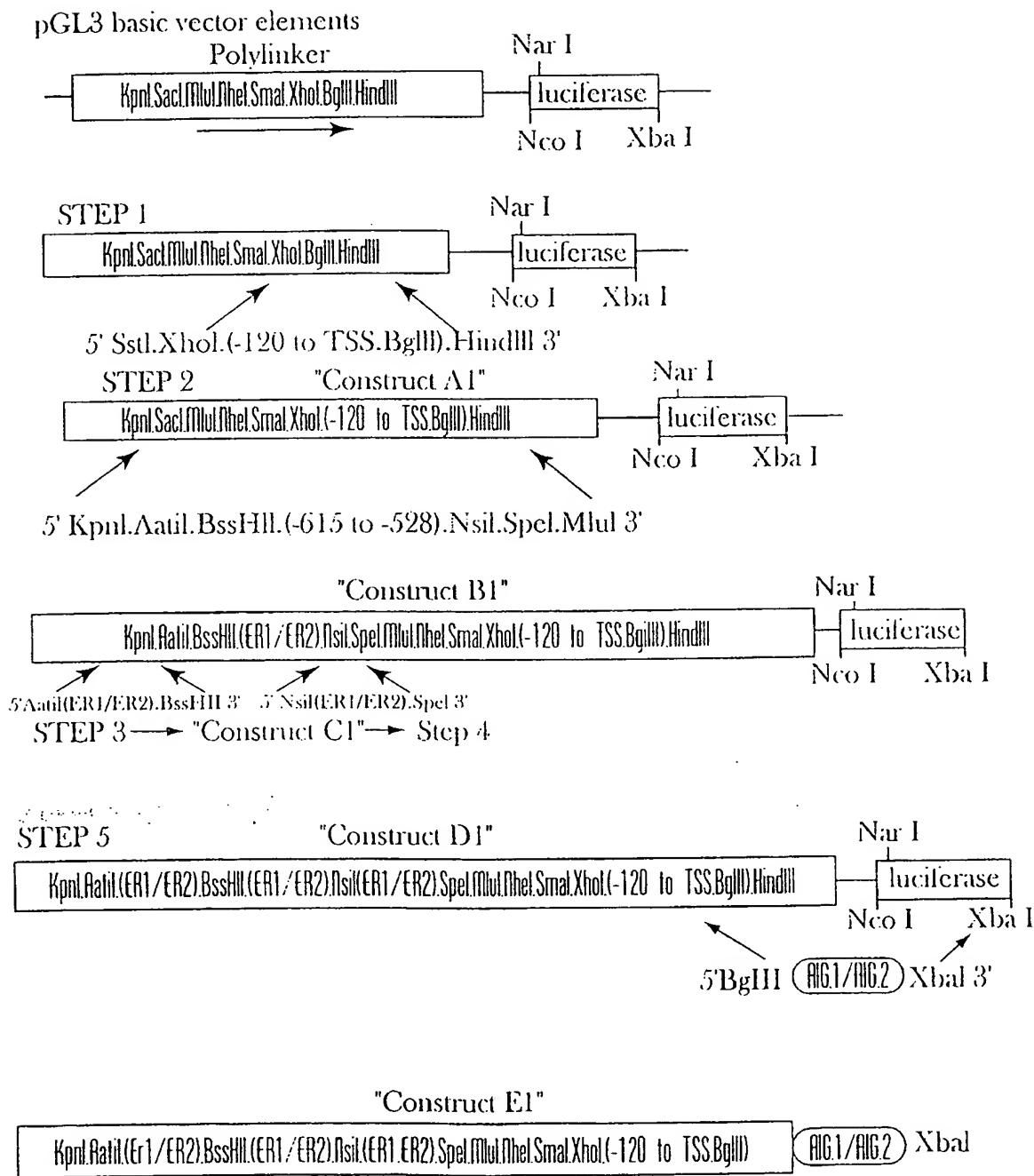


Fig. 8a

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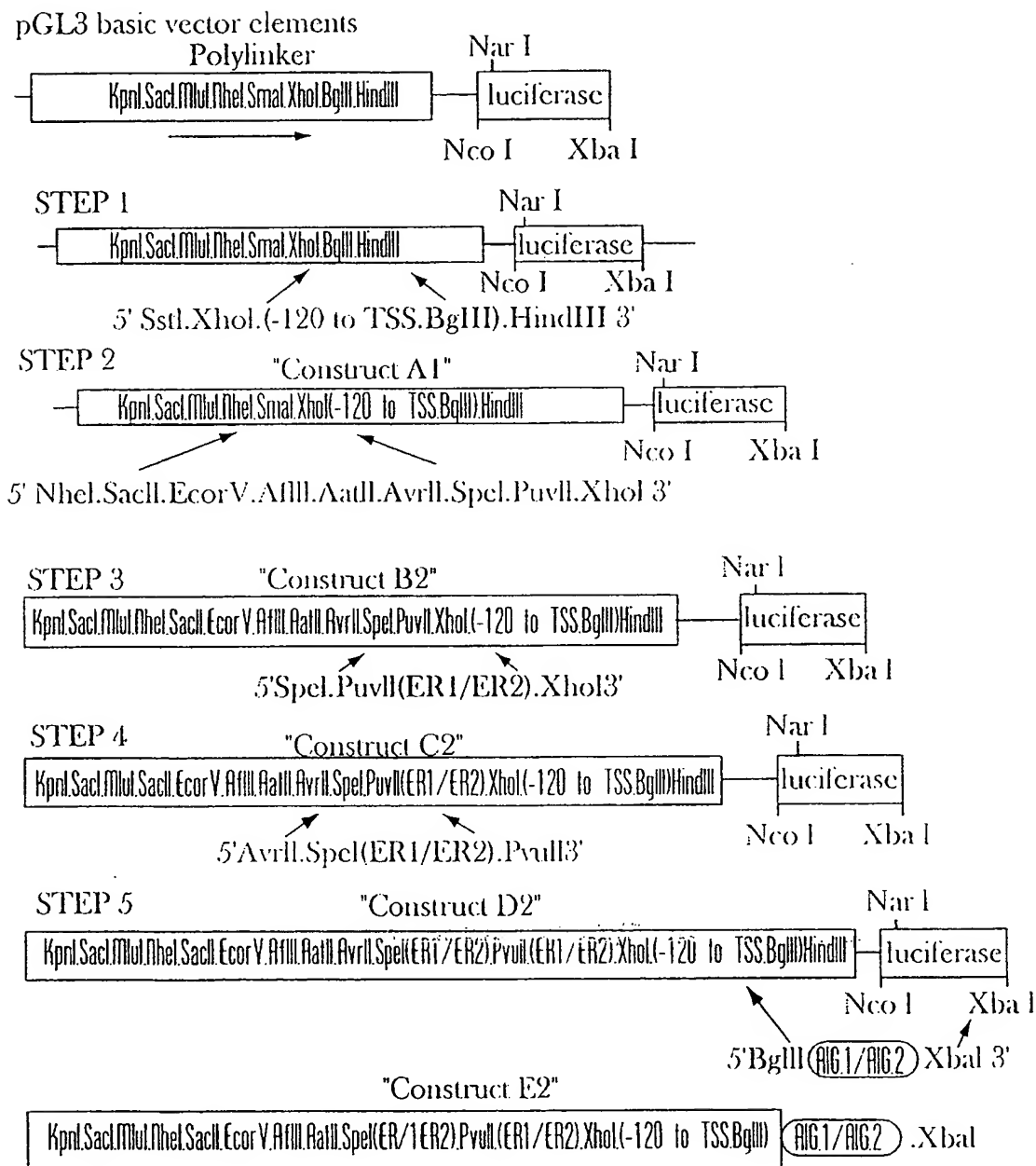


Fig. 8b

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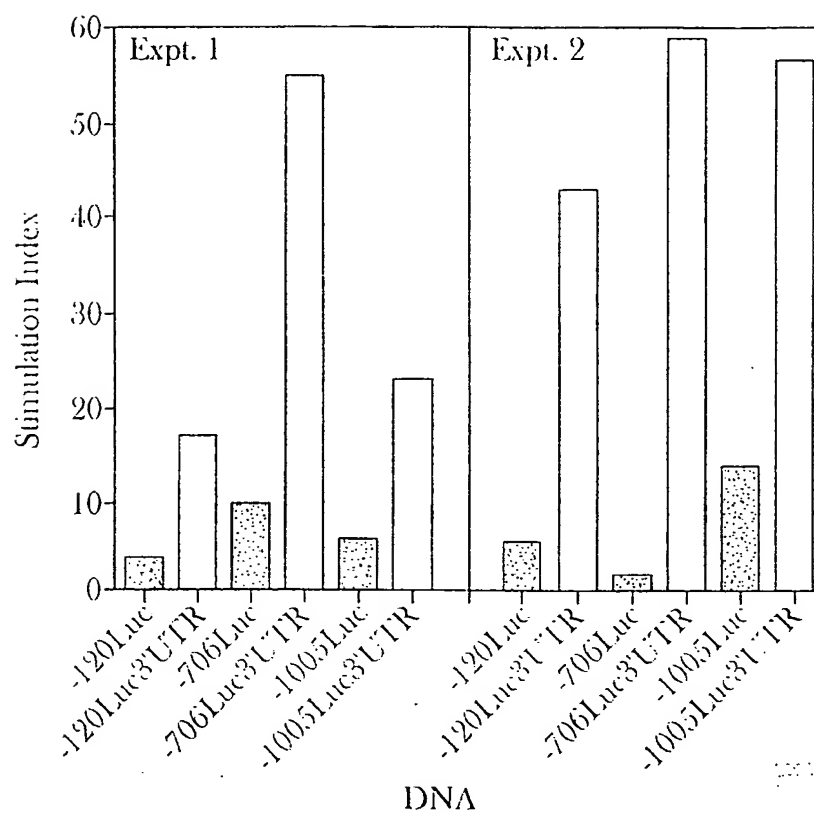


Fig. 9

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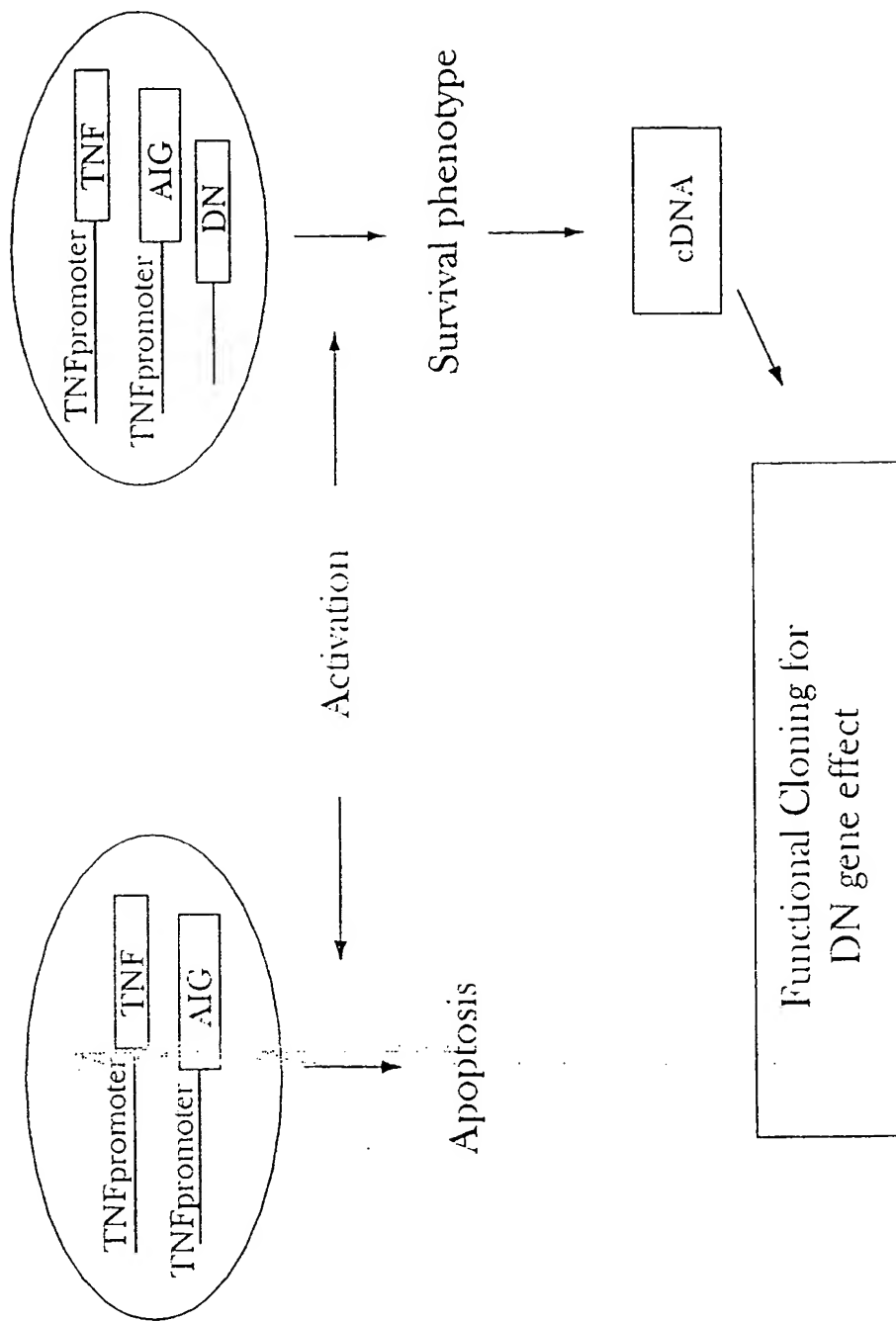


Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/03911

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/70; C07H 21/04; C12N 15/12; C12P 19/34

US CL : 435/91.2; 514/44; 536/23.5, 24.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 91.2; 514/44; 536/23.1, 23.5, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RHOADES et al. The regulation of the human tumor necrosis factor alpha promoter region in macrophage, T cell, and B cell lines. J. Biol. Chem. 05 November 1992, Vol. 267, No. 31, pages 22102-22107, see entire document.	1-35
Y,E	US 5,744,304 A (MUNFORD) 28 April 1998, columns 3-6.	1-35
A	ANDERSON, G.P. Resolution of chronic inflammation by therapeutic induction of apoptosis. Trends Pharmacol. Sci. December 1996, Vol. 17, No. 12, pages 438-442, see entire document.	1-35

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 MAY 1998

Date of mailing of the international search report

23 JUN 1998

 Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03911

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BELLAMY et al. Cell death in health and disease: the biology and regulation of apoptosis. Sem. Cancer Biol. February 1995, Vol. 6, No. 1, pages 3-16, see entire document.	1-35

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/03911

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN: MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS, JAPIO, PATOSEP, PATOSWO

APS

Search Terms: apoptosis, programmed cell death, inflammation, TNF alpha, cytokine, promoter, enhancer